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Oxysterol Receptors LXRs and Coregulators in Cholesterol Metabolism and Inflammatory Transrepression Pathways

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To my Mother

ABSTRACT

The LXRs are important sensors and regulators of cholesterol homeostasis in several metabolic tissues including liver, intestine and macrophages. They regulate target genes involved in cholesterol-, lipid- and carbohydrat metabolism. Recently, the LXRs have emerged as important regulators of the innate and adaptive immune system and inflammation. In this thesis we have extend the knowledge of the specific coregulator requirement of LXR in cholesterol metabolism and the anti-inflammatory actions of LXR and LRH-1 in the hepatic acute phase response. Moreover, we also suggest that LXRs protects against the development of colitis.

In Article I we show that RAP250 has a critical role in the canonical TGF-β pathway and interacts with the intracellular mediators, Smad2 and Smad3. The interaction between RAP250 and Smad2/3 is dependent upon the second LXXLL motif in RAP250 and the MH2 domain in Smad2/3. Moreover, activation of the TGF-β and LXR signaling pathways synergistically regulates the expression of the LXR target gene ABCG1. Thus, the cross talk between LXR and TGF-β could play an important role in the cholesterol efflux pathway.

In Article II we demonstrate that LXR and the transcriptional co-regulator GPS2 mediates promoter specific induction of ABCG1 expression and subsequently increased cholesterol efflux from macrophages. GPS2 is selectively required for LXR induced transcription of ABCG1 and depletion of GPS2 diminishes ABCG1-mediated cholesterol efflux in macrophages. GPS2 and LXR authorise histone 3 lysine 9 demethylation-coupled activation of ABCG1. Activation and recruitment of LXR to regulatory LXR binding sites (LXRE) in the ABCG1 gene induce a communication between the promoter and the enhancer LXRE in the ABCG1 gene. Additionally, LXR and GPS2 interactions appear to be AF-2 independent, thus separated from the classical LXXLL interaction domain of common LXR transcriptional co-regulators.

In Article III we show that LXRs and LRH-1 dampened the hepatic acute phase response. This was due to ligand dependent SUMOylation of LXR and LRH-1, which further prevented the dissociation of the NCoR corepressor complex where GPS2 mediates the interaction between SUMOylated NRs and the NCoR corepressor complex. GPS2 binds to SUMO-1 and SUMO-2 via a domain located in the N-terminal part of GPS2, suggesting that SUMOylated LXR and LRH-1 bind to the corepressor complex via docking to GPS2 and this interaction depends on both the SUMO molecule and the receptor. Moreover, transrepression by LXR appears to exclude the heterodimeric partner RXR and *in vivo* data suggest that LXRβ selectively inhibits hepatic APR.

In Article IV we report that the LXRs protect against DSS-induced colitis in mice. Clinical markers of colitis including weight reduction, colon length and diarreha were significantly more severe in LXR$\beta^{-/-}$ and LXR$\alpha\beta^{-/-}$ mice compare to the wild type (WT) control mice. Further, LXR$\alpha\beta^{-/-}$ mice recovered more slowly from the colitis symptoms compared to WT mice. Activation of LXR in human colon cells under inflammatory conditions repressed the expression of several pro-inflammatory factors and LXR is recruited to the promoter of inflammatory genes. The above- mentioned data could be the reason for the increased infiltration of macrophages seen in LXR KO mice and the more severe immune response to DSS treatment in LXR KO compared to WT mice.

In summary, our studies have identified novel molecular mechanisms of LXR signaling in metabolism and inflammation. Modulation of LXR activity affects the expression profiles of both metabolic pathways and inflammatory signaling pathways. Our observations support the notion that LXRs are attractive drug targets for therapeutic intervention of metabolic disorders and inflammatory diseases.

LIST OF PUBLICATIONS

I. Antonson, P., **Jakobsson, T**., Almlof, T., Guldevall, K., Steffensen, K. R., and Gustafsson, J. A. (2008). *RAP250 is a coactivator in the transforming growth factor beta signaling pathway that interacts with Smad2 and Smad3.* Journal of Biological Chemistry (2008), 283, 8995-9001.

II. **Jakobsson, T**., Venteclef, N., Toresson, G., Damdimopoulos, A. E., Ehrlund, A., Lou, X., Sanyal, S., Steffensen, K. R., Gustafsson, J. A., and Treuter, E. *GPS2 is required for cholesterol efflux by triggering histone demethylation, LXR recruitment, and coregulator assembly at the ABCG1 locus*. Molecular Cell (2009), 34, 510-518.

III. Venteclef, N., **Jakobsson, T**., Ehrlund, A., Damdimopoulos, A., Mikkonen, L., Ellis, E., Nilsson, L. M., Parini, P., Janne, O. A., Gustafsson, J. A., Steffensen, K. R., and Treuter, E. *GPS2-dependent corepressor/SUMO pathways govern anti-inflammatory actions of LRH-1 and LXRβ in the hepatic acute phase response*. Genes & Development (2010), 24, 381-395.

IV. **Jakobsson, T**., Vedin, L., Venteclef, N., Treuter, E., Gustafsson, J. A., and Steffensen, K. R. *The oxysterol receptors, LXRα and LXRβ, protect against DSS-induced colitis in mice.* Manuscript.

Related Publications:

Jakobsson, T., Osman, W., Gustafsson, J. A., Zilliacus, J., and Warnmark, A. (2007). *Molecular basis for repression of liver X receptor-mediated gene transcription by receptor-interacting protein 140.* Biochemical Journal (2007)**,** 405, 31-39.

Venteclef, N., **Jakobsson, T.**, Steffensen, K. R., and Treuter, E. *Metabolic nuclear receptor signaling and the inflammatory acute phase response.*
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LIST OF ABBREVIATIONS

Aβ	Amyolid β
ABC	ATP-binding cassette
ACAT	Acyl-CoA: cholesterol acyltransferase
AF-1/2	Activation function-1/2
AP-1	Activator protein 1
Apo	Apolipoprotein
APP	Acute phase proteins
APR	Acute phase response
AR	Androgen receptor
ASCOM	ASC-2 complex
C/EBP	CCAAT-enhancer binding protein
CAR	Constitutive androstane receptor
CCR7	CC chemokine receptor-7
CD	Crohn´s disease
CEH	Cholesteryl ester hydrolase
CETP	Cholesterol ester transfer protein
CoRNR-box	Corepressor nuclear receptor-box
CORO2A	Coronin 2A
COX-2	Cyclooxygenase-2
CRP	C-reactive protein
Cyp7a1	Cholesterol 7α-hydroxylase
DAD	Deacetylase-activating domain
DBD	DNA binding domain
DC	Dendritic cell
ER	Estrogen receptor
FAS	Fatty acid synthase
FXR	Farnesoid X receptor
G6Pase	Glucose 6-phosphatase
GLUT4	Glucose transporter 4
GPS2	G-protein pathway suppressor 2
GR	Glucocorticoid receptor
HDACs	Histone deacetylases
HDL	High density lipoproteins
HNF4α	Hepatocyte nuclear factor
HRE	Hormone response elements
IBD	Inflammatory bowel disease
IL	Interleukin
iNOS	Inducible nitric oxide synthase
JNK1	C-jun N-terminal kinase 1
LBD	Ligand binding domain
LCAT	Lechitin: cholesterol acyltransferase
LPS	Lipopolysaccaride
LRH-1	Liver receptor homologue-1
LXR	Liver X receptor

LXRE	Liver X receptor response element
NCoR	Nuclear receptor corepressor
NF-κB	Nuclear Factor-κB
NMR	Nuclear magnetic resonance
NOD	Nucleotide oligomerisation domain
NR	Nuclear receptor
PAI-1	Plasminogen activator inhibitor-1
PEPCK	Phosphoenolpyruvate carboxykinase
PGC-1α	Peroxisome proliferator activated receptor gamma co-activator α
PLTP	Phospholipid transfer protein
PPAR	Peroxisome proliferator-activated receptor
PXR	Pregnane X receptor
RAP250	Nuclear receptor-activating protein 250
RAR	Retinoic acid receptor
RCT	Reverse cholesterol transport
RD	Repression domain
RIP140	Receptor interacting protein 140
RXR	Retinoid X receptor
SAA	Serum amyloid a
SAP	Serum amyloid p
SCD1	Stearoyl-CoA desaturase 1
SHP	Small heterodimer partner
Smad	Derived from the Sma and MAD gene homologues
SMRT	Silencing mediator of retinoic acid and thyroid receptor
SR-BI	Scavenger receptor class BI
SREBP-1c	Sterol regulatory element-binding protein 1c
STAT	Signal transducer and activator of transcription
SUMO	Small ubiquitin-like modifier
TBLR1/TBL1	Transducin β-like proteins
TGF	Transforming growth factor
Th	T helper cells
TLR	Toll-like receptors
TNF	Tumor necrosis factor
TopoIIβ	Topoisomerase IIβ
TR	Thyroid receptor
TRRAP	Transformation/transcription domain-associated protein
UBL	Ubiquitin-like modifiers
UC	Ulcerative colitis
VDR	Vitamin D_3 receptor
WT	Wild type

1 INTRODUCTION

1.1 NUCLEAR RECEPTORS

The nuclear receptor (NR) family is a large family of ligand induced transcription factors comprising 48 members in humans. Compounds such as thyroid hormones, steroids, fatty acids and cholesterol derivates regulate gene expression by binding to, and thereby, activating the receptors. NRs regulate several vital processes in the body such as development, metabolism, cell growth and reproduction and numerous pathological conditions are linked to these receptors such as atherosclerosis, hyperlipidemia, obesity, insulin resistance and type 2 diabetes (Francis et al. 2003). The NR superfamily can be divided into six subfamilies according to functional and sequence homologies. The largest subfamily (NR1) includes the liver X receptors (LXR), peroxisome proliferator-activated receptors (PPAR), thyroid receptors (TR), retinoic acid receptors (RAR) and the RAR-related orphan receptors (ROR), among others. NR subfamily 1 group H includes LXRα (NR1H3), LXRβ (NR1H2), FXRα (NR1H4), FXRβ (NR1H5) and the ecdysone receptor (NR1H1), which is found in arthropods (Gronemeyer et al. 2004; Germain et al. 2006).

The mechanisms of action of NRs are in principle similar but there are some variations. The ligand can be generated differently; it may be synthesized within the cell, generated from a pro-hormone or a precursor within the cell or derived from an endocrine organ and transported in the bloodstream to the target cell. Most NRs can both activate and inhibit target gene expression and various classical steroid receptors are in an inactivate state associated with heat shock proteins and dissociate from the complex upon ligand binding and translocate in to the nucleus and bind to DNA at specific sequences called hormone response elements (HREs). A subset of NRs is assumed to be constitutively nuclear with the NR bound to the HRE on the DNA and associated with corepressors in the absence of ligand. Upon binding of ligand the repressor complex dissociates from the receptor and different coactivator complex are recruited in order to activate the target gene expression. Inhibition of target genes by ligand induced NRs involves different tethering mechanisms (no direct DNA binding) and several NRs have been shown to transrepress inflammatory target genes (Pascual and Glass 2006).

NRs display a distinct domain organization and specific functions can be assigned to certain sequences within the receptor. A variable N-terminal A/B domain, which usually contains an activation function 1 (AF-1), a highly conserved C domain, with two ”zinc fingers” creating the DNA binding domain (DBD), a D domain including the hinge region, which is very flexible, thus allowing for rotation of the DBD, a multifunctional E domain, which mediates ligand binding (LBD), activation function 2 (AF-2) and a dimerization surface, and, finally, the F region with the C-terminal tail (Giguere 1999). The binding of coregulators to NRs is often mediated via interactions through AF-1 and AF-2. Most studies have focused on the interactions through AF-2, which targets several coregulators in a ligand dependent fashion via a signature motif (NR-box) displayed on the protein. In contrast, proteins that bind to AF-1 do not share any common motif (i.e. NR-box) (Warnmark et al. 2003). Today numerous crystal structures of the LBD and DBD of NRs have been elucidated. The LBD shares an overall structure with 12 α helices (H1-H12) and one antiparallel β-turn. Upon binding of ligand, the receptor undergoes a conformational change from *apo* (no ligand) to *holo* (liganded) form. This change mainly involves H12 (core of AF-2), which leans over the ligand cavity and seals it upon ligand binding. It is mainly held in place by hydrophobic interactions, which are indispensable to create an appropriate surface that is required for efficient interaction with different coregulators binding to AF-2 (Bourguet et al. 2000; Egea et al. 2000).

1.2 THE LIVER X RECEPTORS

The liver X receptors, LXRα (NR1H3) and LXRβ (NR1H2) generally function as permissive heterodimers with Retinoid X Receptor (RXR). LXRα and LXRβ bind to a specific DNA sequence, called LXR response element (LXRE), which consists of direct repeats of the consensus halfsite sequence 5′-AGGTCA-3′ where the halfsites are spaced by four nucleotides (DR-4 motif) (Zelcer and Tontonoz 2006). LXRα was originally cloned by Apfel et al., with high expression in liver, kidney, intestine, macrophages and adipose tissue (Apfel et al. 1994; Willy et al. 1995). LXRβ was isolated and characterised by several groups the same year and is ubiquitously expressed (Shinar et al. 1994; Song et al. 1994; Teboul et al. 1995). Human LXRα (447 amino acids) and LXRβ (460 amino acids) share 77% sequence homology in the DBDs

and LBDs and structural studies of the LBD of the LXRs have revealed a canonical NR structure (Bourguet et al. 1995; Renaud et al. 1995; Bourguet et al. 2000; Farnegardh et al. 2003; Williams et al. 2003). The natural endogenous ligands for LXRs are oxysterols, which are oxidized derivates of cholesterol, including 24(S)-hydroxycholesterol, 22(R)-hydroxy-cholesterol and 24(S), 25-epoxycholesterol (Janowski et al. 1999; Shibata and Glass 2010). Through structure activity relationship studies there are also synthetic ligands for the LXRs available today, such as T0901317 (N-(2,2,2-tri-fluoroethyl)-N-[4-(2,2,2-tri-fluoro-1-hydroxy-1-tri-fluoro-methyl-ethyl)-phenyl]-benz-enesulfon-amide), GW3965, Acetyl-podocarpic dimer (APD), N-acylthiadiazolines (LXRβ-selective) and sulfonamide GSK2033 (antagonist) (Schultz et al. 2000; Collins et al. 2002; Sparrow et al. 2002; Chao et al. 2008; Zuercher et al. 2010). The best characterised and used synthetic ligands include GW3965 and T0901317.

The LXRs are key regulators of cholesterol metabolism, displaying anti-atherogenic properties, through regulation of various proteins involved in cholesterol transport from peripheral tissues to the liver. Studies also highlight the importance of these receptors in hepatic lipogenesis by induction of fatty acid and triglyceride biosynthesis (mainly via LXRα) through direct regulation of the sterol regulatory element-binding protein 1c (SREBP-1c), fatty acid synthase (FAS) and stearoyl-CoA desaturase 1 (SCD1). Activation of LXR also reduces the expression of several enzymes involved in hepatic gluconeogenesis such as, glucose 6-phosphatase (G6Pase), fructose 1,6-bisphosphatase, phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate carboxylase, thus improving the glucose tolerance. Additionally, the glucose transporter 4 (GLUT4) is regulated by LXRs in white adipose tissue, stimulating the uptake of glucose in this tissue. The anti-atherogenic action of LXR is most likely linked to the emerging anti-inflammatory properties associated to these receptors. In addition, growing amount of data also suggest additional roles for LXRs in the innate and adaptive immune response.

The impact of LXR and coregulators in cholesterol metabolism and inflammation will be covered in further detail below.

1.3 CHOLESTEROL METABOLISM

1.3.1 HDL metabolism

It is well established today that high density lipoproteins (HDLs) display anti-atherogenic properties and presumably anti-inflammatory functions through various complex mechanisms (Rader 2006). The main function of HDL is transport of lipids in the blood, which involves several complex steps. Formation of HDL is initiated through synthesis of apoA-I from the liver and intestine, which subsequently undergoes lipidation (addition of cholesterol and phospholipids), via ATP-binding cassette transporter A1 (ABCA1) expressed on the enterocytes and hepatocytes, forming lipid poor HDL particle. From the peripheral tissues, lipid poor HDL promotes efflux of free cholesterol through ABCA1 and subsequently the mature HDL particle is formed via ABCG1. Maturation of HDL requires esterification of cholesterol through the enzyme lechitin: cholesterol acyltransferase (LCAT). The mature HDL particle is returned to the liver and uptake of cholesterol is mediated through the scavenger receptor class BI (SR-BI). Alternatively, cholesterol is transferred to LDL particles through the cholesteryl ester transfer protein (CETP) and taken up by the liver via binding of the LDL particles to the hepatic LDL receptor (LDLR). In the liver cholesterol is hydrolysed to free cholesterol, which is converted to bile acids or secreted to the bile duct (Rader 2006; Rader and Daugherty 2008).

1.3.2 The ABC-transporters

The ATP-binding cassette (ABC) transporters are a family of transmembrane proteins, which comprise of more than 250 proteins and transport different substrates through the cell membrane such as, bile acids, sterols, phospholipids and peptides, in a process requiring hydrolysis of ATP. The efflux of cholesterol from cells is regulated by the transporters ABCG1, ABCA1, ABCG4, ABCG5 and ABCG8, all regulated by LXR (Baldan et al. 2009). The importance of ABCA1 in cholesterol metabolism was shown in 1999 in patients with Tangier disease. These patients have mutations in the ABCA1 gene and exhibit little or no plasma HDL (Brooks-Wilson et al. 1999; Marcil et al. 1999; Rust et al. 1999). Moreover, ABCA1 deficient mice accumulate cholesterol in macrophages and have increased expression of pro-inflammatory cytokines when challenged with LPS (Zhu et al. 2008). The human ABCG1 (Chen et al. 1996; Croop et

al. 1997) is expressed in many cell types and was originally shown to be involved in lipid transport from macrophages (Klucken et al. 2000). Recently, several groups have shown that ABCG1 together with ABCA1 is important for efflux of cholesterol from macrophages to mature HDL particles (Wang et al. 2004; Kennedy et al. 2005; Wang et al. 2007; Yvan-Charvet et al. 2007). The ABCG5/G8 transport cholesterol from the liver to the bile duct and from the intestine limiting cholesterol uptake from the diet. Cholesterol and plant sterols are absorbed in the intestine via the Niemann Pick C1-like protein (NPC1L1) and the majority of cholesterol is esterified in the enterocytes, whereas some cholesterol and plant sterols are extruded back to the intestinal lumen through ABCG5/G8. Mutations in ABCG5/G8 can cause sitosterolemia, which is a genetic disorder characterised by increased levels of plant sterols, due to an impaired regulation of sterols in the intestine and liver (Oram and Vaughan 2006). The importance of ABCG5/8 in cardiovascular disease has been shown using ABCG5/8 transgenic mice expressing high levels of G5 and G8, which display reduced plasma cholesterol levels and aortic lesion area upon western diet (Wilund et al. 2004).

In addition to the well-known function of ABCA1 and ABCG1 in development of atherosclerosis, it has become evident that these transporters have roles linked to the inflammatory response. Several studies have shown that ABCG1 and ABCA1 deficient mice display enhanced inflammatory response when challenged with LPS highlighting the importance of these transporters in the above-mentioned pathways (Yvan-Charvet et al. 2007).

1.4 LXR AND CHOLESTEROL METABOLISM

The LXRs are important sensors and regulators of cholesterol homeostasis in several metabolic tissues including liver, intestine and macrophages and regulate a variety of target genes encoding diverse enzymes involved in cholesterol synthesis, metabolism, absorption, and transport (Zelcer and Tontonoz 2006). Early *in vivo* evidence demonstrated the importance of the LXRs in cholesterol metabolism using LXR$\alpha^{-/-}$ mice, which when fed with high cholesterol diet accumulated large amounts of cholesterol in the liver due to impaired regulation of the enzyme cholesterol 7α-hydroxylase (Cyp7A1), the rate-limiting enzyme converting cholesterol to bile acids (Peet et al. 1998). This phenotype is restricted to LXR$\alpha^{-/-}$ and is not seen in LXR$\beta^{-/-}$

mice (Alberti et al. 2001). Shortly after, it was recognized that LXR has anti-atherogenic potential by inhibiting the progression and even promoting the regression of atherosclerosis (Joseph et al. 2002; Schuster et al. 2002; Tangirala et al. 2002), which has later been established in several different mouse models (Calkin and Tontonoz 2010).

Subsequent reports have demonstrated regulation of several LXR target genes involved in cholesterol metabolism. The cholesterol ester transfer protein (CETP), which facilitates transfer of cholesterol esters from mature HDL to apoB containing lipoproteins and clearance via the liver, is positively regulated by LXR both *in vitro* and *in vivo* in transgenic mice expressing human CETP (Honzumi et al. 2010; Luo and Tall 2000). LXREs have been identified and characterised in the proximal promoter of phospholipid transfer protein (PLTP) and shown to be functional using T0901317 in macrophage cell cultures (Mak et al. 2002). Moreover, Lafitte et al. have demonstrated that both PLTP and apolipoprotein E (apoE) are regulated in peritoneal macrophages by LXR activation and that the expression of both PLTP and apoE is reduced in LXR deficient animals (Laffitte et al. 2001; Laffitte et al. 2003).

Besides the liver, the intestine is important for synthesis of apoA-I and generation of lipid poor HDL particles via efflux of phopholipids and cholesterol. Recently, using mice lacking ABCA1 in the intestine, Brunham et al. reported that approximately 30% of the plasma HDL is derived from the intestine and that ABCA1 is an important factor involved in HDL biogenesis (Brunham et al. 2006a). Moreover, activation of LXR in mice lacking hepatic ABCA1 significantly raises plasma HDL, thus supporting the idea that intestinal ABCA1 is important for biogenesis of plasma HDL (Brunham et al. 2006b). Lo Sasso et al, recently presented data using constitutively activated LXRα in the intestine, which reduced the cholesterol absorption and increased the levels of lipid poor HDL in mice feed atherogenic western diet. Moreover, these mice also displayed antiatherogenic effects, revealed by reduced atherosclerotic lesions (Lo Sasso et al. 2010). In addition to regulating the expression of ABCA1 in the intestine, LXR also regulates the expression of ABCG5 and ABCG8 in the intestine and the liver. Activation of LXR in mice, and likely in humans (Jiang et al. 2008), up regulates the expression of ABCG5/8 and downregulates the expression of NPC1L1, consequently increasing the sterol secretion from the liver and decreasing the absorption of cholesterol in intestine, thus regulating the efflux of cholesterol (Lo Sasso et al. 2010; Repa et al. 2002; Yu et al. 2003).

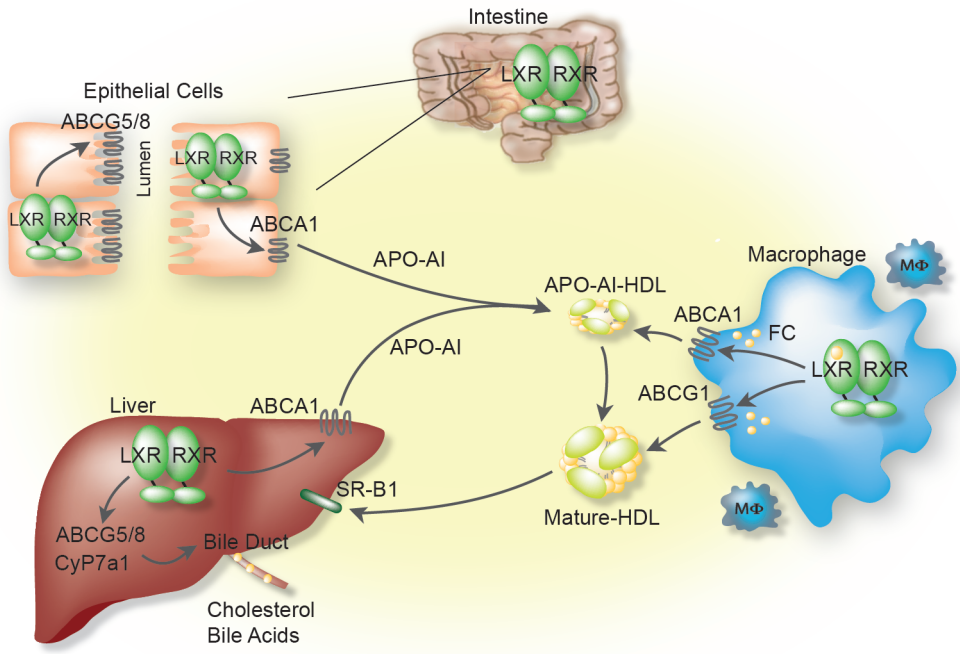

Figure 1. *Role of LXRs in HDL metabolism and cholesterol efflux. Activation of LXR increases the expression of the cholesterol-transporters ABCA1 and ABCG1 in macrophages, subsequently increasing the efflux of cholesterol from macrophages, a process known as reverse cholesterol transport. ABCA1 transports cholesterol to lipid-poor APO-AI-HDL and ABCG1 to mature HDL. HDL is transported to the liver via SR-B1 and in the liver LXR promotes the excretion of cholesterol, via ABCG8/5 and conversion to bile acids through regulation of Cyp7a1 (in rodent), to the bile duct. Activation of LXR also promotes the transport of APO-AI, via upregulation of ABCA1 in the liver and intestine, to generate APO-AI-HDL. In the intestine LXR limits the uptake of cholesterol through activation of ABCG8/G5.*

One of the major efflux pathways from macrophages and a well established protective mechanism against atherosclerosis is the transport of cholesterol to the liver (reverse cholesterol transport (RCT)) via ABCA1 and ABCG1, both regulated by LXR and this pathway has been studied intensively. (Costet et al. 2000; Repa et al. 2000; Schwartz et al. 2000; Venkateswaran et al. 2000; Kennedy et al. 2001; Kennedy et al. 2005). Notably, activation of LXR *in vivo*, using different mouse models, has been shown to increase the rate of RCT from macrophages via ABCG1 and ABCA1 (Naik et al. 2006; Wang et al. 2007; Yvan-Charvet et al. 2007). Additionally, a recent study reported that an intestinal specific LXR agonist promotes RCT in macrophages *in vivo*,

thus emphasizing the importance of LXRs in both intestine and macrophages (Yasuda et al. 2010). Hematopoietic stem cell (HSC)-based LXRα-gene delivery into macrophages in $LDLR^{-/-}$ mice fed western diet appears to reduce the atherosclerotic lesions as well as plasma triglyceride levels and inflammatory cytokines (Li et al. 2011), again highlighting the importance of selective activation of LXR in extrahepatic tissues, to circumvent increased levels of triglycerides in liver and plasma.

1.5 THE IMMUNE SYSTEM AND INFLAMMATION

1.5.1 The innate and the adaptive immune response

The body's defence against microbes and other foreign substances is mediated by the early response called innate immunity and subsequently the slower adaptive response. The innate immunity or "the first defence" is rapid and consists of 4 different components: 1/ the epithelial barrier, 2/ circulating effector cells (neutrophils, monocytes (macrophages) and natural killer (NK) cells), 3/ cytokines and 4/ the acute phase response including the complement system (Rosen 2000). The function of the effector cells is to detect the microbes and through secretion of cytokines stimulate the inflammatory response. The detection of pathogens is mediated via pattern recognition receptors (Toll-like receptors (TLRs)) and others, which detect various molecular patterns including bacterial DNA, lipoproteins, lipopolysaccharide (LPS) and viral DNA (Ishii et al. 2008). The major source of cytokines in the innate immunity is neutrophils, macrophages and NK cells. The expression of cytokines is a complex network of pleiotropic effects, to ensure diverse effects in the host. Most often this response is rapid and transient and operates both systemically and locally.

The adaptive immunity (acquired immunity) is mediated by lymphocytes, so called B cells and T cells (cytotoxic and helper). In response to recognition of different antigens the T cytotoxic cells become activated and the antigen presenting cells are removed by lysis or apoptosis. In addition to T cytotoxic cells, T helper cells become activated during the acquired response and release cytokines for subsequent activation of other cell types involved in the immune response. The B cells produce antibodies targeting antigens for subsequent phagocytosis and activate the complement system. A

small fraction of the B cells becomes memory cells in order to respond quickly and produce antibodies if the same antigen re-infects the host (Rosen 2000).

1.5.2 TGF-β signaling

The cytokine transforming growth factor β (TGF-β) is a pleiotropic factor and regulates a diverse set of cellular responses, such as cell proliferation, apoptosis and different immunomodulatory functions (Li et al. 2006) and dysregulation of TGF-β is linked to several diseases, such as atherosclerosis, cancer and different fibrotic diseases (Blobe et al. 2000). TGF-β binds to surface receptors and the canonical signaling cascade includes phosphorylation and the subsequent activation of Smad transcription factors (Sequence similarity to Sma (*C.elegans)* and Mad (*Drosophila*) proteins). In addition, TGF-β also activates several protein kinase pathways (Massague et al. 2005).

Recent *in vitro* and *in vivo* studies have shown that activation of the TGF-β pathway appears to be anti-atherogenic (Robertson et al. 2003), via increased cholesterol efflux, among other mechanisms, through upregulation of ABCA1 and ABCG1 (Argmann et al. 2001; Panousis et al. 2001).

Given the plethora of effects, not surprisingly, crosstalk between the TGF-β pathway, Smad dependent and independent, and NR signaling has been described for several receptors, such as GR, ER and the PPARs (Song et al. 1999; Matsuda et al. 2001; Stockert et al. 2011).

1.5.3 The acute phase response

The acute phase response (APR) is a systemic reaction that is initiated by local or systemic disturbance caused by infection, inflammation, surgery, trauma and neoplasia. The function of the APR is to prevent microbial growth, restore homeostasis and promote healing. Upon the APR several pro-inflammatory cytokines are released, mainly from monocytes and macrophages, including interleukin 6 (IL-6), inteleukin 1β (IL-1β) and tumor necrosis factor α (TNFα) (Moshage 1997; Gabay and Kushner 1999). The overall aim of the cytokines is to stimulate production of acute phase proteins (APPs), which are primarily produced in hepatocytes and regulated via pro-inflammatory factors such as signal transducer and activator of transcription (STAT) 3,

CCAAT-enhancer binding protein (C/EBP) β, activator protein (AP) 1 and nuclear factor kappa B (NFκB). However, certain APPs are produced in adipose tissues under low-grade inflammatory conditions (e.g. obesity) (Yang et al. 2006; Poitou et al. 2009; Zhao et al. 2010). During the acute phase response, positive and negative APPs are rapidly synthesised and transported to peripheral tissues and the main positive APPs include serum amyloid A (SAA), c-reactive protein (CRP), haptoglobin and plasminogen activator inhibitor-1 (PAI-1). The function of most APPs has not fully been elucidated, however the above-mentioned APPs play important roles in different metabolic changes during APR. The first described APP was CRP (Tillett and Francis 1930), known to activate the complement system and its ability to recognize pathogens. SAA is an apolipoprotein like protein and participates in cholesterol metabolism and induces cytokine production in endothelial cells. Moreover, chronically elevated levels of SAA in adipose tissue have been linked to obesity. Haptoglobin has anti-inflammatory properties and binds haemoglobin, and like SAA is linked to cholesterol metabolism (Gruys et al. 2005).

During the APR major metabolic changes, such as cholesterol and triglyceride synthesis and alterations in HDL associated cholesterol transport occurs (Khovidhunkit et al. 2004). The plasma levels of HDL decrease and the major apolipoprotein of normal HDL (apolipoprotein AI) is replaced by SAA. The acute phase HDL (SAA-HDL) has different composition compared to normal HDL and is believed to be pro-atherogenic and the altered properties of acute phase HDL have significant impact on cholesterol transport and cholesterol efflux (Feingold and Grunfeld 2010; Jahangiri 2010; King et al. 2010). Several studies indicate that SAA-HDL impairs cholesterol efflux (Banka et al. 1995; McGillicuddy et al. 2009; Annema et al. 2010), however there are contradictory reports showing that SAA-HDL increases cholesterol efflux due to enhanced acceptor capabilities of acute phase HDL (Tam et al. 2005; Tam et al. 2008). One explanation for the increased efflux capability of acute phase HDL might involve the regulation of acyl-CoA: cholesterol acyl transferase (ACAT) and cholesteryl ester hydrolase (CEH) by SAA-HDL. Studies have shown that SAA suppresses the expression of ACAT and stimulates the expression of CEH in cholesterol-laden macrophages, consequently, reducing the pool of cholesterol esters and increasing the pool of free cholesterol available for transport to HDL acceptors via ABC-transporters (Tam et al. 2002; Kisilevsky and Tam 2003).

A recent series of investigations has identified liver receptor homolog-1 (LRH-1) as a negative regulator of the hepatic APR (Venteclef et al. 2006; Venteclef and

Delerive 2007). Overexpression of LRH-1 in hepatocytes was reported to result in a strong inhibition of both IL-6 and IL-1β-stimulated expression of SAA, haptoglobin, and fibrinogen. Negative regulation appeared to involve direct transcriptional crosstalk of LRH-1 with C/EBPβ, in part via inhibition of DNA-binding. In addition to LRH-1, PPARs were early linked to the hepatic APR (Gervois et al. 2001; Kleemann et al. 2004; Zambon et al. 2006; Mansouri et al. 2008). Both PPARα and δ are expressed in hepatocytes where they regulate multiple pathways involved in fatty acid and glucose metabolism. Direct transcriptional interference with pro-inflammatory factors such as NFκB and C/EBP has been suggested as a plausible mechanism. A notable exclusion is the glucocorticoid receptor (GR), which acts anti-inflammatory in immune cells but is a positive modulator of APR gene expression in the liver (Wang et al. 2001).

1.5.4 Inflammatory bowel disease

Inflammatory bowel disease (IBD) includes Crohn´s disease (CD) and ulcerative colitis (UC) and the causes behind IBD are believed to be environmental factors, the luminal flora and genetic predisposition (Loftus 2004). Moreover, studies have shown that increased permeability of the epithelial layer to some extent might contribute to the progress of IBD (Hermiston and Gordon 1995; Buhner et al. 2006). The inflammatory regions include ileum, colon and rectum and are most often accompanied by development of mucosal ulceration together with infiltration of a complex mixture of both innate and adaptive immune cells (Abraham and Cho 2009). The infiltration of the immune cells is mediated via TLRs expressed on the epithelium and nucleotide oligomerisation domain (NOD) proteins expressed in dendritic cells and epithelial cells (Rakoff-Nahoum et al. 2004; Strober et al. 2006; Vijay-Kumar et al. 2007). Activation of TLR and NOD increases the activity of NFκB which in turn increases the expression of numerous cytokines and chemokines such as IL-10, IL-6, TNF-α, IL-1β, IL-12, IL-23, IL-8 and TGF-β. This response activates the adaptive response, which includes different subsets of T helper cells (Th1, Th2 and Th17) and their differentiation is determined by different cytokines (Hue et al. 2006; Abraham and Cho 2009). Historically, IBD have been viewed as a T-cell driven disease but increasingly more evidence also suggest important functions of the innate response.

The NRs are becoming an attractive alternative for treatment of inflammatory conditions as several of the NRs have been identified as potent anti-inflammatory

factors. Many studies have reported that different NRs protects against IBD in mice. PPARγ was shown to suppress development of dextran sulfate sodium (DSS) induced colitis in mice and trinitrobenzene sulfonic acid (TNBS) induced colitis in rats (Ramakers et al. 2007; Sanchez-Hidalgo et al. 2007). Interestingly, the protective function of PPARγ appears to be mediated by its anti-inflammatory role in both colonic epithelium (Adachi et al. 2006) and macrophages associated with colitis-induced inflammation (Shah et al. 2007b). Likewise, activation of the farnesoid X receptor (FXR) protects against DSS and TNBS induced colitis in mice; a mechanism also due to suppression of inflammatory responses. However, it is unclear if lack of FXR increases the predisposition to colitis as one report supports this (Vavassori et al. 2009) while the other does not (Gadaleta et al. 2011). Activation of the pregnane X receptor (PXR) was shown to ameliorate DSS-induced colitis in mice, apparently via repression of the NF-κB signaling pathway (Shah et al. 2007a). Heterozygous knock out mice for the LRH-1 had increased inflammatory response to TNBS-induced colitis, which was due to its transcriptional regulatory mechanisms of genes involved in glucocorticoid production in the intestine (Coste et al. 2007).

1.6 LXR AND THE IMMUNE SYSTEM

In addition to the well-known functions of LXR in lipid metabolism, the LXRs have expanded their repertoire and emerged as important factors in the innate and adaptive immune system and inflammation (Zelcer and Tontonoz 2006). The anti-inflammatory activities of LXR were first described 2003 using a cutaneous inflammatory mouse model, in which activation of LXR using GW3965 or 22-hydroxycholesterol inhibited the expression of TNFα and IL-1α (Fowler et al. 2003). The first evidence linking cholesterol metabolism and the innate immunity through LXR were described the same year. These studies provided evidence using LPS stimulated macrophages, that ligand activated LXR inhibits the expression of several inflammatory factors, such as interleukin 6 (IL-6), cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS). Moreover, they also showed that activation of TLR (TLR3/4) signaling compromised several LXR target genes, such as ABCA1 and ABCG1, and consequently the cholesterol efflux via the transcription factor interferon-regulatory factor 3 (IRF3), connecting cholesterol and innate immune signaling to LXR

function (Castrillo et al. 2003; Joseph et al. 2003). The transrepressive mechanism behind the anti-inflammatory action of LXR and other nuclear receptors as well, was originally described by Glass and co-workers (Ricote et al. 1998; Pascual et al. 2005) and will be discussed in further detail below (*1.7.2.1*). Studies have also shown that LXR regulates macrophage survival. Using bone marrow derived macrophages from LXR deficient mice infected with different bacterial pathogens, it has been shown that these cells are more susceptible to bacterial infection and exhibit accelerated apoptosis. Moreover, activated LXR induces the expression of several anti-apoptotic factors, such as AIM, Bcl-X_L and Birc1a and inhibits several proapoptotic factors including several caspases, consequently preventing bacterial induced apoptosis (Joseph et al. 2004; Valledor et al. 2004).

A role in host defence has recently been described for LXR in the lungs. Using different *in vivo* models several groups have shown that activation of LXR in lung tissue macrophages reduces the inflammatory response, induced by LPS and *Klebsiella pneumoniae*, and diminishes the influx of neutrophils to the lungs (Birrell et al. 2007; Smoak et al. 2008). The anti-inflammatory response of LXR might depend on the pathogen, as suggested by a group using LXR deficient mice infected with *Mycobacterium tubercolosis.* This study revealed that these mice were more susceptible to infection compared to WT animals and exhibited a defective neutrophil response (Korf et al. 2009).

Several data also suggest additional functions of LXR in dendritic cells and T-cells, thus linking LXR both to innate and adaptive immunomodulatory functions. LXR activation has been described to hamper LPS induced human myeloid dendritic cell (DC) maturation and function, which in turn blocks the ability of DC to activate T-cells (Geyeregger et al. 2007). Recently, using different tumor models, one study showed that both human and mouse tumors produces endogenous LXR ligands, which inhibit the expression of CC chemokine receptor-7 (CCR7) on maturing DC, thus impairing the antigen presenting function to T- and B-cells. Furthermore, viral knock down of LXRα in human hepatocarcinoma cell lines prevents CCR7 inhibition by 22-hydroxycholesterol. Collectively, this data suggests that that activation of LXR in DC inhibits the antitumor immune response via a reduced migration to lymphoid organs and reduced activation of the adaptive response (Villablanca et al. 2010).

Activated LXR also appears to have anti-proliferative effect in T-cells, which seems to be coupled to the cellular levels of sterols and regulation of the LXR target gene ABCG1. During T-cell proliferation, expression of the enzyme sulfotransferase

family cytosolic 2B member 1 (SULT2B1) is increased, which consequently inactivates the endogenous LXR ligands in T-cells and downregulates several LXR target genes including ABCG1. The downregulation of ABCG1 seems to be a key component given the fact that LXR in lymphocytes from ABCG1 deficient mice displays reduced capability to inhibit proliferation compared to WT mice (Bensinger et al. 2008).

The hallmark of the neuro-inflammatory disease, Alzheimer's disease, is deposition of amyolid β (Aβ) plaques; the formation of Aβ decreases during cellular cholesterol depletion. Not surprisingly, given the known function of LXR linked to inflammation and cholesterol metabolism, several groups have suggested important roles for LXR in Alzheimer's disease. Activation of LXR, *in vitro* and *in vivo*, using T0901317 in neural cells and APP23 transgenic mice has been shown to decrease the levels of Aβ through upregulation of the LXR target gene ABCA1. Moreover, Tangier disease derived fibroblasts lacking functional ABCA1 have increased formation of Aβ, thus linking activation of LXR via ABCA1 to decreased levels of Aβ (Sun et al. 2003; Koldamova et al. 2005). Above-mentioned studies where recently confirmed using LXR deficient mice (Genetic loss of *Lxra* or *Lxrb* in APP/PS1 mice), which displayed increased levels of amyolid plaques compared to WT mice. Furthermore, several LPS induced inflammatory factors in primary microglial cells were repressed with GW3965 and this anti-inflammatory effect increased the phagocytic capacity of these cells, suggesting an increased clearence of Aβ (Zelcer et al. 2007).

Most studies have primarily focused on the anti-inflammatory actions of LXR in different cell-types of the immune system. In addition, ligand activated LXR displays anti-inflammatory actions in the liver, repressing the expression of APPs, including CRP and SAP (Blaschke et al. 2006), although the mechanism behind this effect is unknown.

1.7 NUCLEAR RECEPTORS AND COREGULATORS IN TRANSCRIPTIONAL REGULATION

1.7.1 Transcriptional regulation by NRs

Nuclear receptors recruit a large number of coregulatory proteins in order to regulate transcription. Coregulators function as bridging and/or stabilizing factors, inhibitors of specific protein-protein interactions or modifiers of other proteins with different enzymatic activity, such as acetylation/deacetylation, methylation /de-methylation, SUMOylation, ubiquitylation and phosphorylation. These events initiate (coactivators) or inhibit (corepressors) chromatin remodelling/structure and/or the assembly of the general transcription machinery. Most of the NRs are believed to be constitutively nuclear, except the classical steroid receptors, and the traditional model of NR dependent regulation of transcription (Figure 2) involves a ligand independent interaction with corepressors (e.g. NCoR/SMRT) on DNA. Upon binding of ligand the repressor complex dissociates and different coactivators are recruited (Rosenfeld et al. 2006).

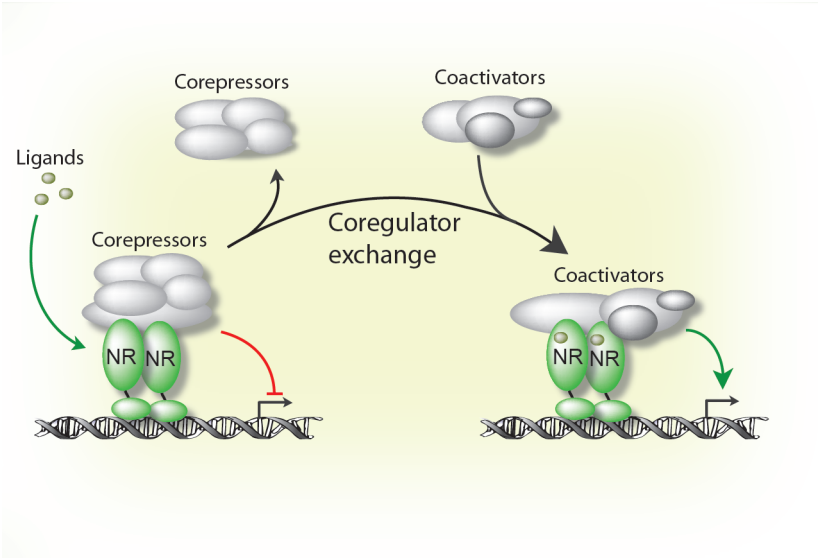

Figure 2. *Classical coregulator exchange mechanism for a subset of NRs. In the absence of ligand the NR is present on DNA with corepressors. Binding of ligand induces a conformational change on the receptor and the subsequent exchange of corepressors for coactivators and activation of target gene expression.*

A well-studied group of coactivators is the p160 family including, steroid receptor coactivator-1 (SRC-1), GR-interacting protein (GRIP1, TIF-2, SRC-2) and p300/CBP cointegrator-associated protein (p/CIP, AIB1, SRC-3) and early studies identified a signature motif (NR-box), LXXLL (L: Leucine; X: any amino acid), found on many NR coactivators, which binds to the AF-2 of ligand activated NRs (Onate et al. 1995; Voegel et al. 1996; Anzick et al. 1997; Heery et al. 1997).

Recent work (epigenomic, genome-wide high-throughput assays) has identified additional NR dependent regulatory mechanisms such as, long-range chromosomal interactions and histone modifications (e.g. histone methylation /demethylation), many of which are dependent on direct or indirect NR-coregulator interactions. Generally, acetylation of histones via histone acetyl transferase (HAT) activity is believed to be an activation mark of transcription, whereas deacetylation via histone deacetylase (HDAC) action is linked to repressive chromatin. Moreover, methylation marks on the histones can be assigned to different functions. For example, methylation of lysine 4 on histone 3 (H3K4) appears to be linked to activation. Furthermore, trimethylation of H3K4 (H3K4me3) is often found at active promoters (often associated with a nucleosome free region), whereas active enhancer regions appear to require mono/di-methylation at H3K4 (H3K4me1 or H3K4me2) (Heintzman et al. 2007; Heintzman et al. 2009). In contrast, methylation of H3K9 induces a repressed state. Methylation and demethylation of histones is executed by methylases and demethylases and these enzymes have been found to coregulate several NRs in transcriptional regulation (Kato et al.). The androgen receptor (AR) has been shown to directly interact with the demethylases KDM1 (LSD1), KDM3A (JHDM2A) and KDM4C (JMJD2C) in a ligand dependent manner, leading to a demethylation of H3K9 and consequently activation of transcription (Metzger et al. 2005; Yamane et al. 2006; Wissmann et al. 2007).

In addition to histone modifications recent studies have also identified long-range chromosomal interactions between NR regulatory elements (i.e promoter-enhancer interactions). It has been suggested that formation of chromatin loops is necessary to activate transcription and that the enhancers are epigenetically marked in order to communicate with the promoter for rapid activation of target genes, although the mechanisms behind these interactions remain unclear (Ong and Corces 2011). Most studies of NRs have primarily focused on the proximal promoters of known NR target genes, however, recent genome wide studies from Brown and co-workers suggest that most binding sites for ER and AR are found distally from promoters in intronic and

intergenic regions (Carroll et al. 2005; Carroll et al. 2006; Lupien et al. 2008), which presumably is true for most NRs. Moreover, many ER distal binding sites are anchored to ER promoters as revealed by a recent global chromatin interaction study, which might function to increase the local concentration of different transcription factors in order to regulate the transcription (Fullwood et al. 2009).

While basic mechanisms of coregulator action are supposed to be preserved between LXRs and other NRs, as revealed by early experiments utilising various interaction and transient transfection assays, the specific coregulator requirement and dynamics of vital LXR target genes remain principally unknown. LXR interacts with several typical NR coregulators, such as the p160 family, receptor interacting protein 140 (RIP140), small heterodimer partner (SHP), NCoR/SMRT, peroxisome proliferator activated receptor γ co-activator α (PGC-1α), transformation/transcription domain-associated protein (TRRAP) and receptor activating protein 250 (RAP250) (Wiebel et al. 1999; Lee et al. 2001; Brendel et al. 2002; Hu et al. 2003; Oberkofler et al. 2003; Huuskonen et al. 2004; Albers et al. 2005; Unno et al. 2005). Although informative, the limitation with transient transfections and interaction studies is that they are performed in a nucleosome free environment and mostly *in vitro*. Wagner et al. addressed this issue using the chromatin immunoprecipitation (ChIP) technique to study the promoter specific roles of LXR in bone marrow derived macrophages, in which they demonstrated that LXR and NCoR/SMRT where recruited to the ABCA1 and SREBP-1c promoters and repressed the gene expression of ABCA1 in the absence of ligand, suggesting promoter specific roles for LXR (Wagner et al. 2003). Moreover, a current study demonstrated that SIRT1 interacts with ligand activated murine LXRs at the ABCA1 promoter and support deacetylation at lysine 433 on LXRβ and 432 on LXRα (corresponding to residue 448 in human LXRβ and 434 in human LXRα). This induces a proteasomal degradation of the receptor and promotes cholesterol efflux through increased LXR activity and this turnover appears to be essential in the activation process as suggested by the authors (Li et al. 2007). To this point, two studies have provided genomic binding sites data governing the LXRβ cistrome, ChIP-seq for biotin-tagged LXRβ in murine macrophages (RAW264.7) and conventional antibody based ChIP-on chip in human epidermal keratinocytes (NHEK). Not surprisingly, most LXRβ binding sites were found distally located to the transcription start site and AP-1 sites were co-enriched close to LXRβ binding sites. Furthermore, in RAW264.7 cells PU.1 sites were co-enriched nearby LXRβ binding sites and PU.1 was suggested to

function as a pioneer factor for LXRβ (Heinz et al. 2010; Shen et al. 2011). Unquestionably, the molecular details of the LXR cistrome/interactome including long-range chromosomal interactions, histone modifications, LXR subtype diversity and tissue/species-specificity (e.g. human macrophages and hepatocytes) are waiting to be discovered in the near future.

1.7.2 Coregulators

1.7.2.1 The NCoR/SMRT complex

Silencing of gene transcription is often linked to deacetylation of histones and this enzymatic process is mediated via recruitment of histone deacetylases (HDACs) to the chromatin. The histone deacetylase HDAC3 belongs to the NCoR or SMRT corepressor complex and these complexes were initially linked to unliganded NR mediated repression, hence the names nuclear receptor corepressor (NCoR) and silencing mediator of retinoic acid and thyroid receptor (SMRT) (Horlein et al. 1995; Alland et al. 1997; Heinzel et al. 1997; Nagy et al. 1997). Unliganded NRs bind to a hydrophobic motif on NCoR/SMRT, so called corepressor nuclear receptor (CoRNR) box (Hu and Lazar 1999; Perissi et al. 1999). In addition to NRs, the NCoR/SMRT corepressor complex can also be recruited to different inflammatory promoters and associate with different transcription factors. Mouse models deficient in either the NCoR or SMRT gene are embryonically lethal (embryonic day 14.5); NCoR appears to be central for the development of erythrocytes and SMRT appears to have important cardiovascular functions (Perissi et al.). The NCoR/SMRT/HDAC3 core corepressor complexes also contain additional proteins including the transducin β-like proteins (TBL1 and TBLR1), G-protein pathway suppressor 2 (GPS2) and coronin 2A (CORO2A). Biochemical studies of the core corepressor complex have revealed direct interactions between TBL1 and GPS2 and cooperative interactions with the N-terminal repression domain 1 (RD1) of NCoR. Moreover, interaction of HDAC3 with NCoR appears to be mediated via a deacetylase-activating domain (DAD) that activates HDAC3 upon binding to the corepressor complex (Guenther et al. 2000; Li et al. 2000; Zhang et al. 2002; Yoon et al. 2003). The interaction between GPS2 (N-terminal) and SMRT (RD1) was recently determined by NMR studies and revealed an antiparallel-coiled coil arrangement, which seems to enhance the binding of these two proteins to the N-teminal region of TBL1. Furthermore, this interaction appears to depend on a

short helical sequence motif (TBL1 interaction motif) found in both GPS2 and SMRT (and NCoR) (Oberoi et al. 2011).

TBLR1/TBL1 have been implicated in corepressor-coactivator exchange mechanisms and suggested to function as adaptor proteins for the recruitment of the ubiquitin conjugating/19S proteasome and the subsequent degradation of the NCoR/ SMRT/HDAC3 proteins, followed by recruitment of coactivators to different genes and this mechanism appears to be dependent on phosphorylated TBLR1/TBL1 (Perissi et al. 2004; Perissi et al. 2008), although the TBLR1/TBL1 dependent exchange mechanism on NR response elements needs further investigation. Moreover, TBLR1/TBL1 bind to hypo-acetylated histones (H2B and H4) and are suggested to be required for stable association of the repression complex with chromatin (Yoon et al. 2003; Yoon et al. 2005)

1.7.2.2 GPS2

The core corepressor subunit GPS2 was initially demonstrated to suppress the RAS/MAPK kinase pathway in mammalian cells via interference with TNFα activated c-jun N-terminal kinase 1 (JNK1) (Spain et al. 1996; Jin et al. 1997). This was confirmed by Roeder and co-workers who provided evidence that a GPS2 containing NCoR/HDAC3 complex inhibited TNFα activated JNK1 (Zhang et al. 2002), although it needs to be clarified if JNK1 inhibition mediated via GPS2 occurs through repression of target genes involved in MAPK signaling or through interference with the canonical MAPK pathway upstream of JNK1. In addition to the inhibitory functions, GPS2 binds to papillomavirus E2 protein and p300 and appears to enhance the transcriptional activity of E2. The differential capacity of GPS2 was further established by interaction studies between p53 and GPS2 (*in vitro* and *in vivo*) demonstrating direct interaction between these proteins and overexpression of GPS2 was shown to significantly affect the transcriptional activity of p53 (Breiding et al. 1997; Peng et al. 2000; Peng et al. 2001). Recently, GPS2 was discovered to display target gene selective activating and repressive functions in liver bile acid biosynthesis. GPS2 were shown to regulate the expression of CYP7A1 and CYP8B1 through interactions with LRH-1, FXR, HNF4α and SHP. On the CYP7A1 promoter GPS2 were found associated with the corepressors NCoR/HDAC3 together with SHP, LRH-1 and HNF4α thus functioning as a repressor. However, on the CYP8B1 promoter GPS2 were found associated with ligand activated FXR, HNF4α and CBP and in contrast to CYP7A1 function as a

coactivator (Sanyal et al. 2007). Subsequent studies have also shown that GPS2 displays important function in ERα signaling. In MCF-7 breast cancer cells it was shown that GPS2 associates with SMRT upon 4-hydroxyl-tamoxifen mediated ERα repression and upon estradiol (E2) treatment on the pS2 promoter. Moreover, depletion of GPS2 in these cells, using siRNA, stimulated cell proliferation, although both in the presence and absence of ERα ligands, indicating that this effect is independent of ERα (Cheng and Kao 2009).

1.7.2.3 RAP250

Receptor activating protein RAP250 (Caira et al. 2000), also known as ASC-2, NCoA6, NRC, PRIP and TRBP (Mahajan and Samuels 2008), was originally isolated as a NR coregulator but has since then emerged as a coregulatory protein for several transcription factors, including CREB, p53 and NF-κB, among others. RAP250 contains two activation domains (AD1 and AD2) and two LxxLL motifs. LxxLL-1 (amino acids 887-891) has been shown to bind to numerous NRs including RAR, TR, ER, PPAR, GR, FXR, PXR, LXR, CAR and RXR. The second LxxLL motif (amino acids 1491-1495) appears to be more restrictive and binds to LXRα, LXRβ and FXR (Ananthanarayanan et al. 2011; Surapureddi et al. 2008; Lee et al. 2001; Mahajan and Samuels 2008; Surapureddi et al. 2008; Kim et al. 2009a). Deletion of RAP250 in mice results in embryonic lethality and could be explained by placental dysfunction and abnormalities in the liver, brain and heart (Kuang et al. 2002; Antonson et al. 2003; Mahajan et al. 2004). Given the specific interaction with LXR via the second NR-box; Kim et al. developed transgenic mice expressing a dominant negative fragment of RAP250, named DN2 (residue 1431-1511 (including LxxLL-2)). Interestingly, on high-cholesterol diet these mice displayed a highly homologous phenotype compared to the described phenotype in LXRα deficient mice, including accumulation of cholesterol in the liver (Kim et al. 2003) and impaired expression of several LXR target genes, including ABCG1 and ABCA1, in macrophages (Kim et al. 2009b). Moreover, recent data suggest that RAP250 recruits the histone H3 lysine 4 methyltransferases MLL3 and MLL4 proteins, subunits of the ASCOM (ASC-2 complex) complex (Goo et al. 2003), to ligand activated LXR target genes, which in turn leads to trimethylation of histone 3 lysine 4 (H3K4me3) (Lee et al. 2008). Furthermore, the ASCOM complex seems to play crucial role in RAR and FXR signaling pathways (Lee et al. 2006; Kim et al. 2009a). In addition to the ASCOM complex, RAP250 co-immunoprecipitates

together with Topoisomerase IIβ, PARP1 and DNAPK, among others proteins, in whole cells extracts from E_2 treated MCF-7 cells. The TopoIIβ-containing complex is suggested to regulate initiation of transcription via dsDNA break formation, hence linking components of the DNA damage and repair machinery to NR mediated gene activation and RAP250 (Ju et al. 2006).

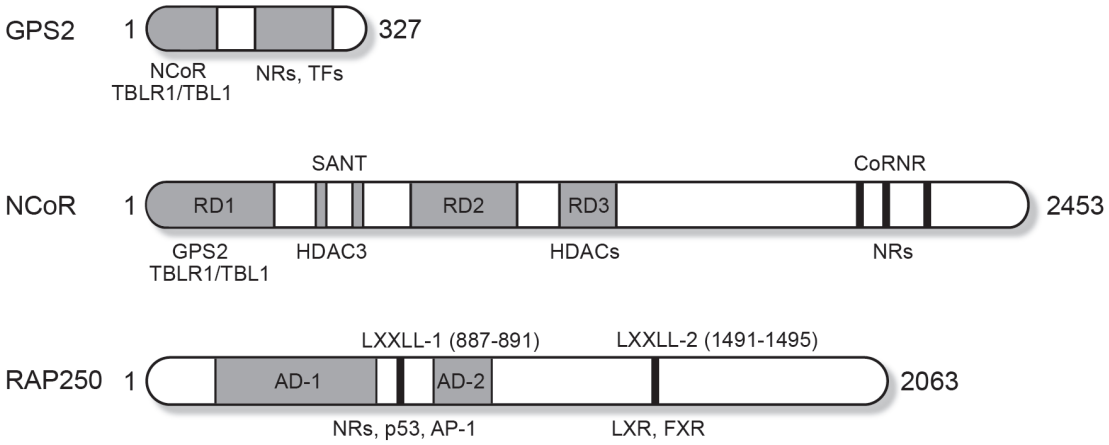

Figure 3. *Schematic picture of important interaction domains in GPS2, NCoR and RAP250. TFs: Transcription factors, CoRNR: Corepressor nuclear receptor, RD: Repression domain, AD: Activation domain, SANT: Swi3-Ada2-NCoR-TFIIIB*

1.7.3 SUMOylation

Post-translational modifications of proteins involves attachments of various molecules and proteins, including among others, methylation, acetylation and covalent attachment of ubiquitin and ubiquitin-like modifiers (UBLs). One UBL, the small ubiquitin-like modifier (SUMO) has been shown to regulate various cellular processes, including DNA repair, replication and transcription via covalent attachment to substrate proteins, hence altering the properties of the substrate, such as the stability and activity. In mammals the SUMO family consists of three paralogues, SUMO-1 and the highly related SUMO-2 and SUMO-3 (Gareau and Lima). The conjugation of SUMO to an acceptor lysine of substrates consists of different enzymatic steps including, ATP-dependent activation of SUMO by a heterodimeric E1 enzyme SAE1/2 (E1), transfer to the conjugating enzyme UBC9 (E2) and subsequent conjugation of the SUMO molecule to the substrate that usually requires an E3 ligase (E3). Several E3 ligases

have been identified, such as the PIAS proteins, RanBP2 and several HDACs (Geiss-Friedlander and Melchior 2007).

The first NR reported to be SUMOylated (SUMO-1) was the androgen receptor (AR) (Poukka et al. 2000), followed by the identification of several SUMOylated NRs, such as ER, PPAR, GR, LRH-1 and LXR. To date, SUMOylation of NRs appears to correlate with decreased transcriptional activity. Many identified SUMO-NRs contain an acceptor lysine within a classic consensus motif ΨKxE (Ψ=large hydrophobic amino acid and K=acceptor lysine). Interestingly, this consensus site is not present in LXRα and LXRβ, which are modified by the SUMO-2/3-HDAC4 pathway, in contrast to most other NRs, which are modified by the SUMO-1-PIAS1 pathway (Treuter and Venteclef 2010; Ghisletti et al. 2007).

1.7.4 Transrepression of NCoR/SMRT

NCoR and SMRT are important regulators of LPS induced primary response genes and appear to be associated to p50 (NF-kB) homodimers (Hargreaves et al. 2009) or c-Jun homodimers (AP1) (Ogawa et al. 2004) under basal conditions. LPS stimuli lead to a p65/IKKε dependent phosphorylation of c-Jun and a TBLR1/TBL1 dependent recruitment of the ubiquitin conjugating/19S proteasome and an exchange of corepressors and recruitment of coactivators to activate inflammatory gene transcription (Huang et al. 2009). The NCoR/SMRT corepressor clearance mechanism has been shown to be prevented by ligand activated NRs, such as LXR and PPARγ via a tethering process known as transrepression (Figure 4). Anti-inflammatory effects via transrepression was early shown to be mediated by the GR, although via different mechanisms compared to LXR and PPARγ (Glass and Saijo). Transrepression of the NCoR/SMRT complex was first identified in mouse macrophages, in which PPARγ was shown to repress LPS induced inflammatory genes in a SUMO dependent pathway (Pascual et al. 2005). Shortly after, LXRα and LXRβ was also identified to repress LPS induced inflammatory genes in macrophages via a parallel mechanism conjugated to SUMO-2/3 by the E3 ligase HDAC4 (see section 1.4.2), whereas PPARγ is conjugated to SUMO1 by the E3 ligase PIAS1 (Ghisletti et al. 2007).

Interestingly, one SUMO acceptor site in LXRβ appears to be at residue 448 and 434 in LXRα, which also is acetylated by SIRT1 (lysine 433 in LXRβ and lysine

432 in LXRα). Moreover, promoter studies of LPS stimulated macrophages have shown that most inflammatory target genes repressed by ligand activated LXRs contain NF-kB and AP-1 sites, but not LXREs, indicating that LXRs bind via protein-protein interactions on TLR4 target genes (Ogawa et al. 2005). In addition to the SUMO-2-HDAC4-LXR pathway in macrophages a recent study suggests that ligand activated LXRβ is SUMOylated by PIAS1 and conjugated to SUMO-1 in IFNγ stimulated brain astrocytes inhibiting STAT-1 mediated inflammatory response (Lee et al. 2009). The above-mentioned data suggest that LXR, and other NRs as well; most likely utilize different strategies in different cell-types to prevent activation of inflammatory genes, although it is still an open question how SUMOylated LXRs dock to the corepressor complex.

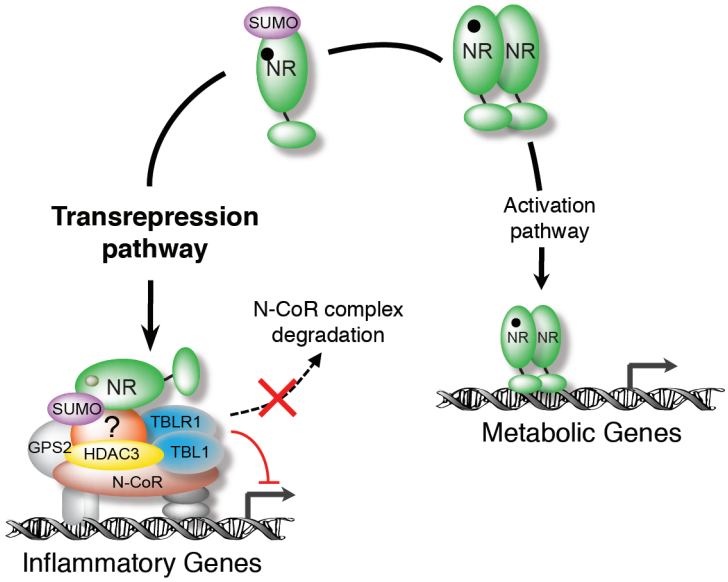

Figure 4. *Anti-inflammatory transrepression by LXRs and PPARs. In addition to the conventional activation pathway, LXR and PPAR become postranslationally modified by SUMO and subsequently enter the transrepression pathway. Transrepression of inflammatory target genes involves docking to the corepressor complex and the thereby inhibition of degradation of the complex.*

2 AIMS OF THESIS

The general aim of this thesis was to characterise the combined influence of LXR in cholesterol metabolism and inflammation. In particular, our intentions were to:

- Investigate the specific coregulator requirement of LXR target genes.
- Understand the selective requirement of GPS2 for the LXR regulated target gene ABCG1 in cholesterol metabolism.
- Investigate the anti-inflammatory action of LXR in the acute phase response and the connection to GPS2 and the core corepressor complex.
- Investigate the role of LXR in colon and its involvement in inflammatory bowel disease.

3 RESULTS

3.1 ARTICLE I: RAP250 IS A COACTIVATOR IN THE TRANSFORMING GROWTH FACTOR BETA SIGNALING PATHWAY THAT INTERACTS WITH SMAD2 AND SMAD3

To identify RAP250 interacting proteins we screened a human ovary library using the yeast two-hybrid system with RAP250 (amino acids 1300-1771) as bait. Among other proteins we isolated clones that encoded the intracellular mediators of the canonical TGF-β signaling pathway, specifically the MH2 domain of Smad2 and its homolog Smad3 (derived from the Sma and MAD gene homologues in *C. elegans* and *D. melanogaster*). Smad2 and Smad3 have a similar structure consisting of an N-terminal mad homology (MH) 1 domain and a C-terminal MH2 domain separated by a linker region (ten Dijke and Hill 2004). Domain mapping in yeast revealed that RAP250 interacted with Smad2/3 through a region between amino acids 1485-1512 and interestingly this region includes the second NR box (NR2) that selectively binds to LXR. Moreover, mutation of NR2 completely abolished the interaction with Smad2. To further investigate the interaction between Smad2 and NR2 of RAP250 we made chimeras of RAP250 NR1 and NR2 interaction motifs, which revealed that in addition to NR2, the C-terminal flanking region was essential. The interaction between Smad2 (MH2) and RAP250 (NR2) was also confirmed in mammalian cells.

To further investigate the biological relevance of this interaction, TGF-β was activated in mouse embryonic fibroblasts (MEFs) isolated from wild type and RAP250 knock-out embryos. Interestingly, the induced mRNA expression of the known TGF-β target genes PA1-1 and connective tissue growth factor (CTGF) was reduced by 50% in RAP250-KO-MEFs compared to RAP250-WT-MEFs. Given the coregulatory function of RAP250 within LXR signaling pathways we also investigated if TGF-β could influence the expression of LXR target genes. We treated human Huh7 liver cells and human U937 macrophages with TGF-β, T0901317 and both in combination and monitored the expression of ABCG1, ABCA1 and SREBP-1c. TGF-β alone had moderate effect on the ABCG1 expression, however co-treatment with TGF-β and T0901317 had a strong synergistic effect compared to T0901317 treatment alone. This effect was not seen at other LXR target genes, suggesting a promoter specific role for TGF-β upon LXR activation on the ABCG1 promoter. The above experimental setup

in LXR-KO-MEFs failed to synergistically induce the expression of ABCG1 whereas this effect was intact in LXR-WT-MEFs.

3.2 ARTICLE II: GPS2 IS REQUIRED FOR CHOLESTEROL EFFLUX BY TRIGGERING HISTONE DEMETHYLATION, LXR RECRUITMENT, AND COREGULATOR ASSEMBLY AT THE ABCG1 LOCUS

Recent work from our laboratory suggests that the core corepressor subunit GPS2 has dual (repressive and activating) functions in cholesterol homeostasis. Focused on this, we applied an unbiased approach and depleted the expression of GPS2 in human hepatic HepG2 cells and THP-1 macrophages to study the impact of GPS2 in LXR signaling pathways.

Interestingly, siRNA mediated knockdown of GPS2 in these cells significantly reduced LXR induced mRNA expression of ABCG1 while not affecting other known LXR target genes (e.g. ABCA1, SREBP-1c). In contrast, depletion of TBLR1, another member of the NCoR corepressor complex, reduced the expression of all LXR target genes in the presence of LXR ligand. Depletion of NCoR or TBL1 did not affect any of the LXR regulated target genes under these conditions. Moreover, protein expression of ABCG1 in macrophages using siRNA against GPS2 confirmed our mRNA expression data. To demonstrate if knockdown of GPS2 was of physiological relevance, we measured the efflux of cholesterol to HDL acceptor (ABCG1 efflux pathway) in macrophages. Ligand activated LXR increased the cholesterol efflux, whereas depletion of GPS2 abolished the increased LXR dependent cholesterol efflux. Collectively, these data indicates that GPS2 is selectively required for LXR mediated expression and activation of ABCG1.

Above observations encouraged us to explore the recruitment of GPS2 in relation to other coregulators at the promoter regions of human ABCG1 and ABCA1 using chromatin immunoprecipitation (ChIP) assays. In the absence of ligand we identified LXR together with the corepressor components; NCoR, HDAC3, TBLR1, TBL1 and GPS2 on the ABCA1 promoter. Activation of LXR caused their dissociation (except TBLR1) and coactivators were recruited (e.g. CBP, SRC-1), which is indicative of a conventional LXR-dependent coregulator exchange mechanism. Intriguingly, on the ABCG1 promoter in the absence of ligand, neither LXR nor GPS2 were present on the

ABCG1 promoter, however, upon ligand treatment both LXR and GPS2 were recruited to the promoter linking GPS2 to transcriptional activation on the ABCG1 promoter. These results were seen in both HepG2 and THP-1 cells and confirmed *in vivo* in mice liver. Moreover, GPS2 was not recruited to the ABCG1 promoter in LXR$\alpha\beta^{-/-}$ mice treated with LXR ligand indicating that binding of LXR is essential for GPS2 recruitment. Collectively, these observations suggest that GPS2 has specific functions linked to transcriptional activation together with LXR on the ABCG1 promoter and these activities appear to be promoter specific and conserved between human and mouse.

In an attempt to elucidate if the above-mentioned effects were mediated via direct interactions between LXR and GPS2 we utilized various direct protein-protein interaction assays. The characterisation revealed a ligand-enhanced interaction of GPS2 with both LXRα and LXRβ, and GPS2 appears to bind a surface distinct from AF-2 in the LXRs. Furthermore, we identified a minimal LXR interacting GPS2 domain (a.a. 150-264) lacking LXXLL motifs.

ABCA1 appears to utilize one LXRE on the promoter upon activation, while ABCG1 in addition to the promoter LXRE, contains an intronic enhancer LXRE (Sabol et al. 2005). To test the functionality of the enhancer we performed time resolving ChIP assays against both LXREs and this revealed that LXR and GPS2 were recruited to both the ABCG1 promoter and enhancer with a nearly identical profile, thus indicating a functional link upon activation. To further substantiate this data we employed chromosome conformation capture (3C) assays, which confirmed that activation of LXR triggers intrachromosomal communication between the promoter and enhancer LXRE. Furthermore, depletion of GPS2 abolished the ligand dependent recruitment of LXR to ABCG1 and the subsequent communication between the promoter/enhancer. In contrast, depletion of GPS2 did not affect the LXR recruitment or coactivator assembly on the ABCA1 promoter.

Initial ChIP data indicated that ABCA1 was acetylated (H3Ac) in the absence of LXR ligand (i.e activation mark) and this was not seen on the ABCG1 promoter. Based on this we employed a time-resolving ChIP assay regarding H3 acetylation and H3K9 dimethylation (i.e. repression mark) on ABCA1 and ABCG1 promoters. Remarkably, on ABCG1 LXR ligand induced a rapid H3K9 demethylation and acetylation, whereas on ABCA1 the repression mark H3K9me2 was absent together with acetylated chromatin (H3Ac). Moreover, we also detected the presence of the methylase G9a in the absence of ligand on the ABCG1 promoter/enhancer and upon activation several

demethylases were recruited (KDM1 (LSD1), KDM3A (JHDM2A) and KDM4A (JHDM3A)). Collectively, these data suggest that the hypoacetylated and hypermethylated (H3K9) chromatin state on ABCG1 could function as a barrier that prevents LXR binding to DNA and upon ligand activation demethylases are recruited to facilitate demethylation and binding of LXR to DNA, a process requiring GPS2.

3.3 ARTICLE III:GPS2-DEPENDENT COREPRESSOR/SUMO PATHWAYS GOVERN ANTI-INFLAMMATORY ACTIONS OF LRH-1 AND LXRβ IN THE HEPATIC ACUTE PHASE RESPONSE

Lipid-sensing NRs appear to play important roles in the inflammatory response and LXR has been shown to inhibit the expression of several LPS induced pro-inflammatory genes in macrophages via a SUMO dependent tethering mechanism, preventing the dissociation of the NCoR/HDAC3 corepressor complex, a mechanism referred as transrepression (Ghisletti et al. 2007). Moreover, ligand activated LXR displays anti-inflammatory actions in hepatocytes (Blaschke et al. 2006), and recently LRH-1 was found to antagonize several cytokines in the liver (Venteclef et al. 2006; Venteclef and Delerive 2007). The link between LXR, GPS2 and the NCoR/HDAC3 corepressor complex, together with our results in article II encouraged us to investigate the mechanism behind the anti-inflammatory actions of LXR and LRH-1 in the acute phase response (APR) in human hepatocytes and mice.

To induce an APR we stimulated human primary hepatocytes with IL-1β and IL-6 and as expected the expression of several APPs was induced. However, pre-treatment with the LXR/LRH-1 ligands (GW3965/GR8470) inhibited the expression of the APPs, except plasminogen activator inhibitor 1 (PAI-1). Furthermore, ChIP assays in Huh7 cells revealed that LXR/LRH-1 were recruited to the NCoR/ HDAC3/GPS2/TBLR1 corepressor complex on the SAA and haptoglobin promoters in ligand treated cells under inflammatory conditions, thus preventing the dissociation of the complex. Additionally, LXR/LRH-1 was not recruited in the absence of ligand under inflammatory conditions indicating that ligand activation induces the recruitment of LXR/LRH-1 to the APR promoters. Interestingly, LXR appears to function in the absence of RXR as observed by our ChIP data.

Next we investigated if recruitment of LXR/LRH-1 was SUMO dependent. Knockdown (KD) studies (siRNA) of SUMO-1 and SUMO-2/3 in Huh7 cells revealed that KD of SUMO-1 affected the transrepressive activity of LRH-1, whereas KD of SUMO-2/3 affected the LXR transrepressive pathway, as previously shown in macrophages. Furthermore, this data was strengthened by the fact that SUMO-2/3 together with LXR and SUMO-1 together with LRH-1, were recruited to the haptoglobin promoter.

To investigate the SUMO dependent transrepression pathway *in vivo*, we treated C57BI/6J (wild type (WT)) and LXR$\alpha\beta^{-/-}$ mice with LPS (+/- GW3925) to induce an inflammatory response. Activation of LXR significantly reduced the mRNA expression of SAA, haptoglobin and CRP in LPS treated mice, which was confirmed at protein level. Importantly, LXR$\alpha\beta^{-/-}$ mice treated with ligand under inflammatory conditions failed to reduce the mRNA expression of APR genes, which prompted us to investigate if both LXR subtypes were capable to transrepress APR genes *in vivo*. Under the same conditions as for LXR WT and LXR$\alpha\beta^{-/-}$, LXR$\alpha^{-/-}$ mice transrepression of APR genes was observed in LXR$\alpha^{-/-}$ mice, whereas no transrepression was seen in LXR$\beta^{-/-}$ mice, thus indicating that LXRβ selectively inhibits hepatic APR, both *in vivo* and *in vitro*. In addition, we also substantiated the mRNA data with ChIP assays from liver samples. As expected, LXR was recruited to the haptoglobin promoter in WT and LXR$\alpha^{-/-}$ mice but was not recruited in LXR$\alpha\beta^{-/-}$ and LXR$\beta^{-/-}$ mice.

Since GPS2 is linked to the NCoR/HDAC3 corepressor complex and in view of our results in article II we investigated the importance of GPS2 in the APR pathway. Using various direct protein-protein assays together with ChIP assays and siRNA transfection we could conclude the following:

- Repression of APR genes requires NCoR and recruitment of LXR or LRH-1 depends on GPS2.
- The N-terminal domain of GPS2 interacts with NCoR and this interaction is crucial for recruitment of LXR and LRH-1.
- GPS2 binds to SUMO-1 and SUMO-2 via a domain located in the N-terminal part of GPS2, suggesting that SUMOylated LXR and LRH-1 binds to the corepressor complex via docking to GPS2 and this interaction depends on both the SUMO molecule and the receptor.

3.4 ARTICLE IV: THE OXYSTEROL RECEPTORS, LXRα AND LXRβ, PROTECT AGAINST DSS-INDUCED COLITIS IN MICE

Given the fact that LXRs could be highly interesting candidates for treatment of inflammatory conditions, supported by their protective role(s) in many inflammatory conditions and diseases, and based upon our findings in article III, we wanted to investigate the role of LXR in colon and its involvement in IBD.

We used DSS-induced colitis, a common model in mice used to study colitis, to examine the role of LXR. We used C57BI/6J mice (wild type (WT)), LXRα$^{-/-}$, LXRβ$^{-/-}$ and LXRαβ$^{-/-}$ mice. WT mice were pre-treated with the synthetic LXR agonist (GW3965) (30mg/kg/day) or vehicle by gavage for 4 days and 2.5% DSS was added in the drinking water (±GW3965) for 9 days and 7 days for LXR KO mice. At day 9 the WT mice had lost 13% of their body weight upon DSS treatment, while no significant effect of GW3965 was observed. The onset of loss of body weight was earlier in LXR KO mice and at day 7 the body wight was reduced 15%, 2% and 14% in LXRαβ$^{-/-}$, LXRα$^{-/-}$ and LXRβ$^{-/-}$ mice. In comparison, only small changes in symptoms and phenotypes were observed in WT mice at day 7.

LXRαβ$^{-/-}$ and LXRβ$^{-/-}$ mice exhibited a severe phenotype including reduced colon length, increased rectal bleeding and diarrhea. These clinical markers were less pronounced in WT and LXRα$^{-/-}$ mice. Histopathological examination of the colon revealed that DSS treatment caused severe ulceration, disruption of crypts and hyperplasia, in all genotypes, although this was more pronounced in LXRαβ$^{-/-}$ and LXRβ$^{-/-}$ mice. Moreover, DSS caused a dramatic increase in infiltration of neutrophil granulocytes and macrophages. Interestingly, infiltration of macrophages was significantly increased in KO mice – particularly in the LXRαβ$^{-/-}$ mice at basal conditions.

Further we investigated the possibility that activation of LXR could influence the weight recovery from colitis. Thus, mice were given 2.5% DSS and upon approximately 10% weight reduction the DSS were withdrawn from water and body weight monitored for 8 days. The GW3965 treated group showed a trend towards a faster recovery of weight compared to untreated animals. The LXRαβ$^{-/-}$ mice recovered significantly slower compared to WT-GW3965 animals. In addition, WT-DSS-GW3965 mice had significantly lower immune response at the endpoint compared to

WT-DSS mice, revealed by lower mRNA expression of several known pro-inflammatory genes, such as IL-6, TNF-α and MCP-1.

The initiation of the innate immune response in IBD involves activation of TLRs and NODs, found on the epithelium, which increases the activity of NF-κB and the subsequent expression of several cytokines and chemokines to attract different immune cells. Given the known transrepressive function of LXR, we treated Colo205 human colorectal adenocarcinoma cells with GW3965 over night followed by TNFα. Interestingly, activation of LXR completely abolished the induced auto-induction of TNFα, reduced the expression of IL-8 by 50% and the expression of caspase-1 by 80%. Moreover ChIP assay on the IL-8 promoter revealed a significant recruitment of ligand activated LXR under inflammatory conditions.

Extraintestinal manifestations are poorly investigated in association with IBD, but is a highly interesting effect of colitis and a recent study reported that reduced levels of hepatic stearoyl-CoA desaturase 1 (SCD1), which is a known LXR target gene, exacerbate colitis in DSS treated mice (Chen et al. 2008). To investigate this we treated WT and LXRαβ$^{-/-}$ mice with 2.5% DSS for 9 and 7 days, respectively, and the protein levels of SAA in plasma were determined using ELISA. DSS increased the SAA levels in all groups, however the response was stronger in LXRαβ$^{-/-}$ mice, compared to WT mice. Moreover, activation of LXR significantly repressed the expression of SAA in the blood in this colitis model in agreement with paper III. We also observed that the reduced expression of SCD1 (and SREBP1c) upon DSS treatment was completely restored by activation of LXR. This suggests that activation of LXRs could prevent extraintestinal symptoms of colitis and therefore have additional roles in protecting against IBD.

4 DISCUSSION

The dual impact of LXRs in inflammatory and metabolic pathways makes them intriguing factors to increase our understanding how metabolic diseases develop an inflammatory component and vice versa. The role of LXR in the cholesterol transport is well established and numerous studies have highlighted the importance of LXR in RCT and the development of cardiovascular disease. In addition, LXRs display anti-inflammatory effects in macrophages and other immune cells, which might contribute to the protective role of LXRs in development of atherosclerosis. The novel functions of LXRs in the innate and adaptive immune response is intriguing and potentially opens up new strategies to treat different chronic inflammatory conditions, such as IBD, arthritis and atherosclerosis. However, one complication towards the development of compounds targeting LXR is the hepatic lipogenic activity, presumably by activated LXRα, which raises the triglyceride levels in the liver and plasma. Consequently, subtype specific compounds that selectively targeting LXRβ have emerged as one possible strategy for drug development. Indeed, $LXRa^{-/-}$ / $apoE^{-/-}$ double knockout mice treated with GW3965 showed reduced atherosclerosis, presumably by an increased activity of ABCA1 and ABCG1 in both intestine and macrophages. In addition, a decreased expression of inflammatory markers was observed in macrophages (Bradley et al. 2007). Moreover, N-acylthiadiazolines have been described as LXRβ selective ligands (Molteni et al. 2007). The anti-inflammatory and immuno-modulatory action of LXRs includes several different cell types in both the innate and adaptive immune response and recent data suggest that in addition to subtype specific compounds, pathway selective (transrepression vs. transactivation) compounds could provide an additional selective strategy (Chao et al. 2008). Intriguingly, some oxysterols appears to function in both pathways whereas some only enters the transactivation pathway (Ghisletti et al. 2007). Collectively, this suggests that in order to develop compounds with a "narrow spectrum" it is crucial to identify the mechanisms (i.e. the interplay between NRs and coregulators) behind these differences in inflammatory and metabolic pathways.

In this thesis we have extend the knowledge of the specific coregulator requirement of LXR in cholesterol metabolism and the anti-inflammatory actions of LXR and LRH-1 in the hepatic APR. Although the effect of LXRs in different transactivation pathways has been intensively studied, the molecular mechanisms still remains largely unexplored. The molecular details by which LXR and other NRs transrepress inflammatory target genes in macrophages was initially described by Glass and colleagues and in this thesis we found that this mechanism also occur outside the immune system, in liver cells. Furthermore, we present *in vivo* data suggesting that in addition to liver cells and macrophages this mechanism, although not studied in detail, also occur in epithelial cells.

In **article I** we identified the intracellular mediators of the canonical TGF-β signaling pathway, Smad2 and Smad3, as RAP250 interacting proteins and this interaction appears to be mediated via the MH2 domain of the Smad proteins and the second NR-box in RAP250. A possible role for RAP250 in the regulation of PAI-1 could be as a linker between CBP/p300 and mediator complexes since both RAP250 and Smad proteins are known to associate with CBP, p300 and the mediator components. Furthermore, using RAP250-KO-MEFs and LXR-KO-MEFs we suggest that RAP250 is a novel coregulator for selected TGF-β target genes and that TGF-β appears to have a pronounced impact on the ABCG1 expression upon LXR activation. RAP250 is a large protein with two LXXLL motifs and has no intrinsic enzymatic activity. RAP250 appears to selectively bind LXR via NR2 and data suggest that RAP250 is an important factor in LXR signaling pathways (Kim et al. 2003). Furthermore, RAP250 is also linked to trimethylation of H3K4 (H3K4me3) (Lee et al. 2008), which is often found at active promoters. This data suggest that RAP250 might function as a scaffold, thus recruiting LXR via NR2 to the ASCOM complex in order to activate transcription.

Recent studies have shown that activation of the TGF-β pathway increases cholesterol efflux through upregulation of ABCA1 and ABCG1 (Argmann et al. 2001; Panousis et al. 2001). Based on our findings, we suggest that this effect partly might depend on a functional LXR pathway. Future studies are required to clarify if this crosstalk is Smad dependent via indirect or direct interactions. Furthermore we do not exclude the possibility that activation of different MAPK pathways through TGF-β is required.

In **article II** we describe major differences between the LXR regulated cholesterol transporters ABCG1 and ABCA1 at the molecular level. We have identified, upon ligand activation, a GPS2 dependent LXR recruitment to the ABCG1 promoter, which potentially could stimulate efforts to develop LXR agonists that target LXR-GPS2 interactions. Such agonists would promote cholesterol efflux via ABCG1 but fail to upregulate the lipogenic genes regulated by LXR, thus overcome the unwanted side-effects such as elevated fatty acid and triglyceride levels. Furthermore, activation and recruitment of LXR induce a communication between the promoter and enhancer of ABCG1. In contrast, on the ABCA1 promoter LXR seems to utilize the conventional LXR-dependent coregulator exchange mechanism. In the absence of ligand LXR is bound to the LXRE on ABCA1 and associated with corepressors. Upon binding of ligand the repressor complex dissociates from LXR and different coactivators are recruited in order to activate the expression of ABCA1. In addition, we also link GPS2 and H3K9 demethylation via recruitment of several demethylases to LXR dependent activation of ABCG1. This could explain the absence of LXR on ABCG1 without ligand i.e. binding of LXR to repressed chromatin is prevented. Most likely, ChIP-seq. studies in the near future will reveal other LXR target genes that utilize a strictly ligand dependent recruitment of LXR as seen on ABCG1 and it will be highly interesting to see if GPS2 co-enriches close to LXR binding sites at these sites.

In **article III** we investigate the anti-inflammatory actions of LXR and LRH-1 in the hepatic acute phase response. During the APR the plasma level of HDL decreases and the major apolipoprotein of normal HDL (apolipoprotein AI) is replaced by SAA. The acute phase HDL (SAA-HDL) is believed to be pro-atherogenic and the altered properties of acute phase HDL have significant impact on cholesterol transport and cholesterol efflux. Interestingly, in article II we link GPS2 to LXR dependent regulation of cholesterol efflux in macrophages and in this study we link the metabolic receptors LXR and LRH-1 to anti-inflammatory pathways in the APR, thus connecting HDL metabolism and inflammation.

Recently, it was shown that SUMOylated LXRs are recruited to inflammatory genes in macrophages (Ghisletti et al. 2007). However, the mechanism behind how SUMOylated LXRs dock to the corepressor complex remained unanswered. Our data indicates that GPS2 might function as a mediator for SUMOylated LXRβ and LRH-1, and other NRs as well, thus answering the “docking” issue. Moreover, we show that

LXR is recruited without the heterodimeric partner RXR. LXR appears to be SUMOylated in the LBD, which theoretically could eliminate the interaction (conformational changes) with RXR, and the established function of LXR as a direct DNA binding factor, thus creating a pool of SUMOylated LXRs primed for the transrepression pathway. Recently, Huang et al. identified an alternative docking mechanism in mouse macrophages, which included a SUMO-LXRβ dependent interaction with CORO2A. Moreover, in addition to the described TBLR1/TBL1 dependent exchange mechanism, the derepression step also appears to involve recruitment of actin through CORO2A, and the subsequent clearance of the corepressor complex from inflammatory genes (Huang et al. 2011). This suggests that the cellular environment is of great importance (i.e. macrophage and liver) and apparently more work is required to identify the mechanisms behind these disparities. Finally, emerging data suggest that there are species differences in terms of anti versus pro-inflammatory functions of LXR and PPARs (Fontaine et al. 2007; Hall and McDonnell 2007), again underscoring the importance to elucidate the mechanisms (i.e. inflammatory stimuli (LPS, TNF-α), ligand specificity, short/long-term treatment) behind the NR dependent transrepression pathways.

In **article IV** we link LXR to anti-inflammatory actions in epithelial cells, which is important components in the inflammatory bowel disease. IBD is a complex disease involving an early innate immune response via epithelial cells, dendritic cells (DC) and macrophages and activation of the NF-κB pathway in epithelial cells via TLRs and NODs is crucial given the connection with the luminal flora. In this study we show that ligand activated LXRs repress several pro-inflammatory factors, including TNFα and IL-8, induced by TNFα in Colo205 epithelial cells and most likely via a related mechanism as in macrophages and in paper III (liver cells), although the molecular mechanisms were not studied in detail. This suggests that LXR has important anti-inflammatory function in epithelial cells and subsequently reduces the recruitment of immune cells via transrepression of cytokines and chemokines. Moreover, in LXRαβ$^{-/-}$ mice a higher content of macrophages was seen in the colon under basal conditions, which partly could be explained by the above-mentioned mechanism. We also found that LXRβ deficient mice displayed a more severe phenotype when challenged with DSS, such as ulceration and rectal bleeding, however this was also seen in LXRα mice, although not that pronounced. This suggests that both subtypes have protective

functions in IBD. Based upon recent findings, we speculate that LXRα might be involved in the innate response, whereas LXRβ is the crucial factor in the acquired response. LXR has important functions in lymphocytes (Bensinger et al. 2008; Geyeregger et al. 2009) and activation of LXR appears to inhibit the differentiation of T-cells via induced transcription of the *Srebp1* target gene (Cui et al. 2011). The fact that IBD has been viewed as a T-cell driven disease also suggests that LXRs are possible targets in the acquired response to prevent the development of IBD. In conclusion, our observations suggest that LXR protects against IBD, through transrepressive mechanisms in the innate response in epithelial cells and potentially, as shown by others, through repressive functions in the acquired response.

In summary, our studies have identified novel molecular mechanisms of LXR signaling in metabolism and inflammation. Modulation of LXR activity affects the expression profiles of both metabolic pathways and inflammatory signaling pathways. Our observations support the notion that LXRs are attractive drug targets for therapeutic intervention of metabolic disorders and inflammatory diseases.

5 CONCLUSIONS

In this thesis we have investigated the specific coregulator requirement and dynamics of LXR target genes in cholesterol metabolism and the anti-inflammatory actions of LXR in liver and colon and the conclusion from these articles can be summarised as follows:

Article I

- RAP250 has a critical role in the canonical TGF-β pathway and interacts with the intracellular mediators, Smad2 and Smad3.
- The interaction between RAP250 and Smad2/3 is dependent upon the second LXXLL motif in RAP250 and the MH2 domain in Smad2/3.
- Activation of the TGF-β and LXR signaling pathways synergistically regulates the expression of the LXR target gene ABCG1.

Article II

- GPS2 is selectively required for LXR induced transcription of ABCG1 and depletion of GPS2 diminishes ABCG1-mediated cholesterol efflux in macrophages.
- There are fundamental differences between ABCG1 and ABCA1 with regard to GPS2 and other coregulators.
- GPS2 and LXR authorise histone 3 lysine 9 demethylation-coupled activation of ABCG1.
- Activation and recruitment of LXR induces a communication between promoter and enhancer elements of ABCG1.
- LXR and GPS2 interactions appear to be AF-2 independent.

Article III

- Ligand dependent SUMOylation of LXR and LRH-1 inhibits the hepatic acute phase response, thus preventing the dissociation of the NCoR corepressor complex.
- GPS2 mediates the interaction between SUMOylated NRs and the NCoR corepressor complex.
- Transrepression by LXR appears to exclude the heterodimeric partner RXR.

- LXR is recruited to APR promoters in LXR WT and LXRα mice, but not in LXRβ mice indicating that LXRβ selectively inhibits hepatic APR.
- GPS2 interacts with NCoR through the N-terminal domain. This domain also binds to SUMO-1 and SUMO-2 and this interaction depends on both the SUMO molecule and LXR/LRH-1.

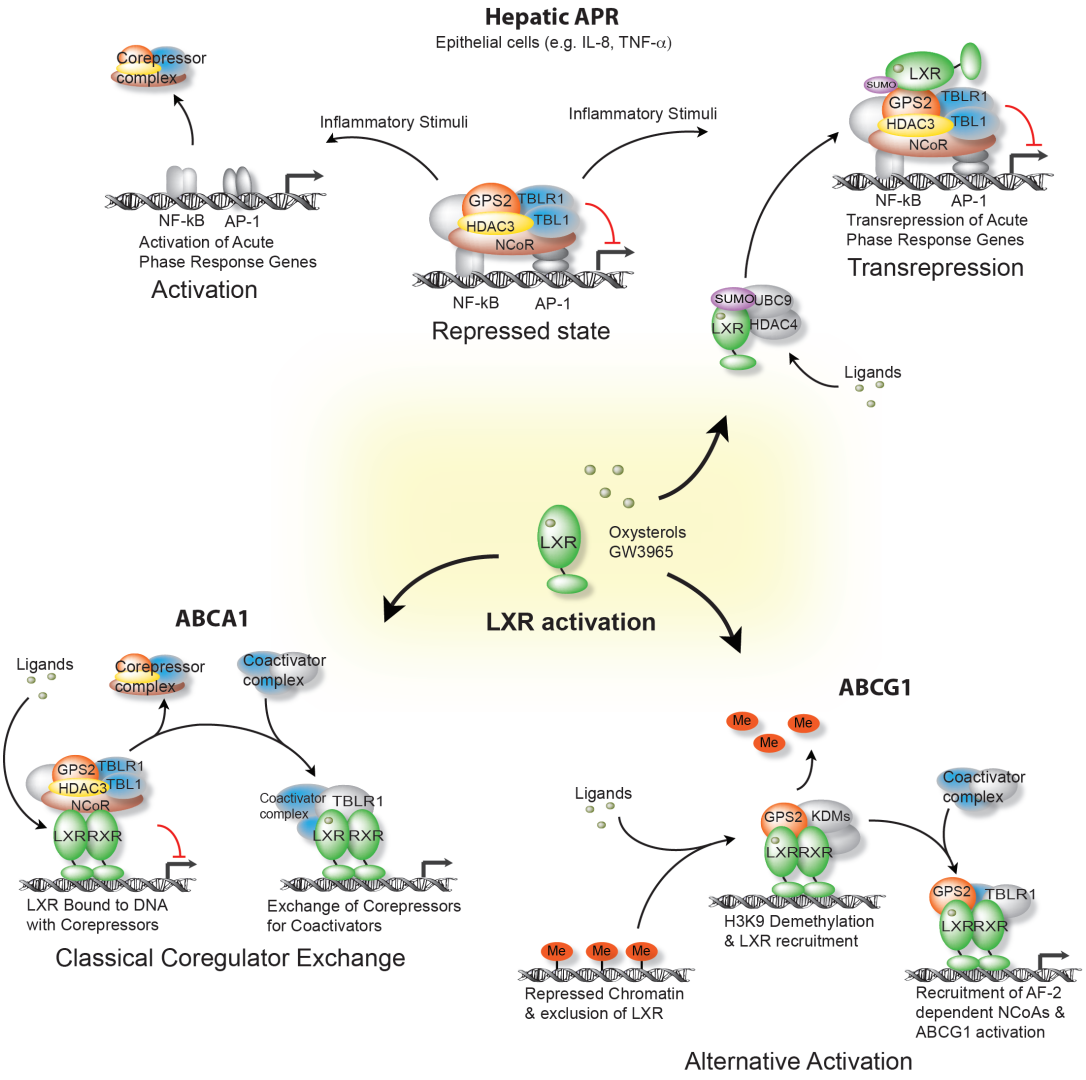

Figure 5. *Schematic picture emphasising the major conclusions in this thesis. In the hepatic APR, LXR-SUMO-2 and LRH-1-SUMO-1 docks to the NCoR corepressor complex via the subunit GPS2 and prevents the clearance of the repressor complex, thereby repressing the expression of APR genes. The anti-inflammatory actions of LXR in epithelial cells are presumably related to this mechanism. In macrophages and liver cells LXR regulates the expression of ABCG1 through an alternative mechanism, which is dependent on GPS2. In contrast, on the ABCA1 promoter LXR utilise the classical LXR dependent coregulator exchange mechanism.*

Article IV

- DSS-induced colitis in C57BI/6J (WT), LXR$\alpha^{-/-}$, LXR$\beta^{-/-}$ and LXR$\alpha\beta^{-/-}$ mice caused a particularly severe phenotype in LXR$\beta^{-/-}$ and LXR$\alpha\beta^{-/-}$ mice, this was also seen in LXR$\alpha^{-/-}$ mice, although not that pronounced.
- DSS recovery studies revealed that LXR$\alpha\beta^{-/-}$ mice recovered significantly more slowly compared to C57BI/6J mice treated with GW3965.
- Activation of LXR in human colon cells under inflammatory conditions repressed the expression of several pro-inflammatory factors and LXR is recruited to the IL-8 promoter.
- The above point could be the reason for increased infiltration of macrophages in LXR KO mice at basal level.
- The increased infiltration of macrophages could be the reason for a more severe immune response to DSS treatment in LXR KO compared to WT mice.

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7 REFERENCES

Abraham C, Cho JH. 2009. Inflammatory bowel disease. *N Engl J Med* **361**: 2066-2078.

Adachi M, Kurotani R, Morimura K, Shah Y, Sanford M, Madison BB, Gumucio DL, Marin HE, Peters JM, Young HA et al. 2006. Peroxisome proliferator activated receptor gamma in colonic epithelial cells protects against experimental inflammatory bowel disease. *Gut* **55**: 1104-1113.

Albers M, Kranz H, Kober I, Kaiser C, Klink M, Suckow J, Kern R, Koegl M. 2005. Automated yeast two-hybrid screening for nuclear receptor-interacting proteins. *Mol Cell Proteomics* **4**: 205-213.

Alberti S, Schuster G, Parini P, Feltkamp D, Diczfalusy U, Rudling M, Angelin B, Bjorkhem I, Pettersson S, Gustafsson JA. 2001. Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXRbeta-deficient mice. *J Clin Invest* **107**: 565-573.

Alland L, Muhle R, Hou H, Jr., Potes J, Chin L, Schreiber-Agus N, DePinho RA. 1997. Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. *Nature* **387**: 49-55.

Ananthanarayanan M, Li Y, Surapureddi S, Balasubramaniyan N, Ahn J, Goldstein JA, Suchy FJ. Histone H3K4 trimethylation by MLL3 as part of ASCOM complex is critical for NR activation of bile acid transporter genes and is downregulated in cholestasis. *Am J Physiol Gastrointest Liver Physiol*.

Annema W, Nijstad N, Tolle M, de Boer JF, Buijs RV, Heeringa P, van der Giet M, Tietge UJ. 2010. Myeloperoxidase and serum amyloid A contribute to impaired in vivo reverse cholesterol transport during the acute phase response but not group IIA secretory phospholipase A(2). *J Lipid Res* **51**: 743-754.

Antonson P, Schuster GU, Wang L, Rozell B, Holter E, Flodby P, Treuter E, Holmgren L, Gustafsson JA. 2003. Inactivation of the nuclear receptor coactivator RAP250 in mice results in placental vascular dysfunction. *Mol Cell Biol* **23**: 1260-1268.

Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM, Meltzer PS. 1997. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science* **277**: 965-968.

Apfel R, Benbrook D, Lernhardt E, Ortiz MA, Salbert G, Pfahl M. 1994. A novel orphan receptor specific for a subset of thyroid hormone-responsive elements and its interaction with the retinoid/thyroid hormone receptor subfamily. *Mol Cell Biol* **14**: 7025-7035.

Argmann CA, Van Den Diepstraten CH, Sawyez CG, Edwards JY, Hegele RA, Wolfe BM, Huff MW. 2001. Transforming Growth Factor-{beta}1 Inhibits Macrophage Cholesteryl Ester Accumulation Induced by Native and Oxidized VLDL Remnants. *Arterioscler Thromb Vasc Biol* **21**: 2011-2018.

Baldan A, Bojanic DD, Edwards PA. 2009. The ABCs of sterol transport. *J Lipid Res* **50 Suppl**: S80-85.

Banka CL, Yuan T, de Beer MC, Kindy M, Curtiss LK, de Beer FC. 1995. Serum amyloid A (SAA): influence on HDL-mediated cellular cholesterol efflux. *J Lipid Res* **36**: 1058-1065.

Bensinger SJ, Bradley MN, Joseph SB, Zelcer N, Janssen EM, Hausner MA, Shih R, Parks JS, Edwards PA, Jamieson BD et al. 2008. LXR signaling couples sterol metabolism to proliferation in the acquired immune response. *Cell* **134**: 97-111.

Birrell MA, Catley MC, Hardaker E, Wong S, Willson TM, McCluskie K, Leonard T, Farrow SN, Collins JL, Haj-Yahia S et al. 2007. Novel role for the liver X

nuclear receptor in the suppression of lung inflammatory responses. *J Biol Chem* **282**: 31882-31890.

Blaschke F, Takata Y, Caglayan E, Collins A, Tontonoz P, Hsueh WA, Tangirala RK. 2006. A nuclear receptor corepressor-dependent pathway mediates suppression of cytokine-induced C-reactive protein gene expression by liver X receptor. *Circ Res* **99**: e88-99.

Blobe GC, Schiemann WP, Lodish HF. 2000. Role of transforming growth factor beta in human disease. *N Engl J Med* **342**: 1350-1358.

Bourguet W, Ruff M, Chambon P, Gronemeyer H, Moras D. 1995. Crystal structure of the ligand-binding domain of the human nuclear receptor RXR-alpha. *Nature* **375**: 377-382.

Bourguet W, Vivat V, Wurtz JM, Chambon P, Gronemeyer H, Moras D. 2000. Crystal structure of a heterodimeric complex of RAR and RXR ligand-binding domains. *Mol Cell* **5**: 289-298.

Bradley MN, Hong C, Chen M, Joseph SB, Wilpitz DC, Wang X, Lusis AJ, Collins A, Hseuh WA, Collins JL et al. 2007. Ligand activation of LXR beta reverses atherosclerosis and cellular cholesterol overload in mice lacking LXR alpha and apoE. *J Clin Invest* **117**: 2337-2346.

Breiding DE, Sverdrup F, Grossel MJ, Moscufo N, Boonchai W, Androphy EJ. 1997. Functional interaction of a novel cellular protein with the papillomavirus E2 transactivation domain. *Mol Cell Biol* **17**: 7208-7219.

Brendel C, Schoonjans K, Botrugno OA, Treuter E, Auwerx J. 2002. The small heterodimer partner interacts with the liver X receptor alpha and represses its transcriptional activity. *Mol Endocrinol* **16**: 2065-2076.

Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HO et al. 1999. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat Genet* **22**: 336-345.

Brunham LR, Kruit JK, Iqbal J, Fievet C, Timmins JM, Pape TD, Coburn BA, Bissada N, Staels B, Groen AK et al. 2006a. Intestinal ABCA1 directly contributes to HDL biogenesis in vivo. *J Clin Invest* **116**: 1052-1062.

Brunham LR, Kruit JK, Pape TD, Parks JS, Kuipers F, Hayden MR. 2006b. Tissue-specific induction of intestinal ABCA1 expression with a liver X receptor agonist raises plasma HDL cholesterol levels. *Circ Res* **99**: 672-674.

Buhner S, Buning C, Genschel J, Kling K, Herrmann D, Dignass A, Kuechler I, Krueger S, Schmidt HH, Lochs H. 2006. Genetic basis for increased intestinal permeability in families with Crohn's disease: role of CARD15 3020insC mutation? *Gut* **55**: 342-347.

Caira F, Antonson P, Pelto-Huikko M, Treuter E, Gustafsson JA. 2000. Cloning and characterization of RAP250, a novel nuclear receptor coactivator. *J Biol Chem* **275**: 5308-5317.

Calkin AC, Tontonoz P. 2010. Liver x receptor signaling pathways and atherosclerosis. *Arterioscler Thromb Vasc Biol* **30**: 1513-1518.

Carroll JS, Liu XS, Brodsky AS, Li W, Meyer CA, Szary AJ, Eeckhoute J, Shao W, Hestermann EV, Geistlinger TR et al. 2005. Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. *Cell* **122**: 33-43.

Carroll JS, Meyer CA, Song J, Li W, Geistlinger TR, Eeckhoute J, Brodsky AS, Keeton EK, Fertuck KC, Hall GF et al. 2006. Genome-wide analysis of estrogen receptor binding sites. *Nat Genet* **38**: 1289-1297.

Castrillo A, Joseph SB, Vaidya SA, Haberland M, Fogelman AM, Cheng G, Tontonoz P. 2003. Crosstalk between LXR and toll-like receptor signaling mediates bacterial and viral antagonism of cholesterol metabolism. *Mol Cell* **12**: 805-816.

Chao EY, Caravella JA, Watson MA, Campobasso N, Ghisletti S, Billin AN, Galardi C, Wang P, Laffitte BA, Iannone MA et al. 2008. Structure-guided design of N-phenyl tertiary amines as transrepression-selective liver X receptor modulators with anti-inflammatory activity. *J Med Chem* **51**: 5758-5765.

Chen C, Shah YM, Morimura K, Krausz KW, Miyazaki M, Richardson TA, Morgan ET, Ntambi JM, Idle JR, Gonzalez FJ. 2008. Metabolomics reveals that hepatic stearoyl-CoA desaturase 1 downregulation exacerbates inflammation and acute colitis. *Cell Metab* **7**: 135-147.

Chen H, Rossier C, Lalioti MD, Lynn A, Chakravarti A, Perrin G, Antonarakis SE. 1996. Cloning of the cDNA for a human homologue of the Drosophila white gene and mapping to chromosome 21q22.3. *Am J Hum Genet* **59**: 66-75.

Cheng X, Kao HY. 2009. G protein pathway suppressor 2 (GPS2) is a transcriptional corepressor important for estrogen receptor alpha-mediated transcriptional regulation. *J Biol Chem* **284**: 36395-36404.

Collins JL, Fivush AM, Watson MA, Galardi CM, Lewis MC, Moore LB, Parks DJ, Wilson JG, Tippin TK, Binz JG et al. 2002. Identification of a nonsteroidal liver X receptor agonist through parallel array synthesis of tertiary amines. *J Med Chem* **45**: 1963-1966.

Coste A, Dubuquoy L, Barnouin R, Annicotte JS, Magnier B, Notti M, Corazza N, Antal MC, Metzger D, Desreumaux P et al. 2007. LRH-1-mediated glucocorticoid synthesis in enterocytes protects against inflammatory bowel disease. *Proc Natl Acad Sci U S A* **104**: 13098-13103.

Costet P, Luo Y, Wang N, Tall AR. 2000. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. *J Biol Chem* **275**: 28240-28245.

Croop JM, Tiller GE, Fletcher JA, Lux ML, Raab E, Goldenson D, Son D, Arciniegas S, Wu RL. 1997. Isolation and characterization of a mammalian homolog of the Drosophila white gene. *Gene* **185**: 77-85.

Cui G, Qin X, Wu L, Zhang Y, Sheng X, Yu Q, Sheng H, Xi B, Zhang JZ, Zang YQ. 2011. Liver X receptor (LXR) mediates negative regulation of mouse and human Th17 differentiation. *J Clin Invest* **121**: 658-670.

Egea PF, Mitschler A, Rochel N, Ruff M, Chambon P, Moras D. 2000. Crystal structure of the human RXRalpha ligand-binding domain bound to its natural ligand: 9-cis retinoic acid. *EMBO J* **19**: 2592-2601.

Farnegardh M, Bonn T, Sun S, Ljunggren J, Ahola H, Wilhelmsson A, Gustafsson JA, Carlquist M. 2003. The three-dimensional structure of the liver X receptor beta reveals a flexible ligand-binding pocket that can accommodate fundamentally different ligands. *J Biol Chem* **278**: 38821-38828.

Feingold KR, Grunfeld C. 2010. The acute phase response inhibits reverse cholesterol transport. *J Lipid Res* **51**: 682-684.

Fontaine C, Rigamonti E, Nohara A, Gervois P, Teissier E, Fruchart JC, Staels B, Chinetti-Gbaguidi G. 2007. Liver X receptor activation potentiates the lipopolysaccharide response in human macrophages. *Circ Res* **101**: 40-49.

Fowler AJ, Sheu MY, Schmuth M, Kao J, Fluhr JW, Rhein L, Collins JL, Willson TM, Mangelsdorf DJ, Elias PM et al. 2003. Liver X receptor activators display anti-inflammatory activity in irritant and allergic contact dermatitis models: liver-X-receptor-specific inhibition of inflammation and primary cytokine production. *J Invest Dermatol* **120**: 246-255.

Francis GA, Fayard E, Picard F, Auwerx J. 2003. Nuclear receptors and the control of metabolism. *Annu Rev Physiol* **65**: 261-311.
Fullwood MJ, Liu MH, Pan YF, Liu J, Xu H, Mohamed YB, Orlov YL, Velkov S, Ho A, Mei PH et al. 2009. An oestrogen-receptor-alpha-bound human chromatin interactome. *Nature* **462**: 58-64.
Gabay C, Kushner I. 1999. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* **340**: 448-454.
Gadaleta RM, van Erpecum KJ, Oldenburg B, Willemsen EC, Renooij W, Murzilli S, Klomp LW, Siersema PD, Schipper ME, Danese S et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. *Gut* **60**: 463-472.
Gareau JR, Lima CD. The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition. *Nat Rev Mol Cell Biol* **11**: 861-871.
Geiss-Friedlander R, Melchior F. 2007. Concepts in sumoylation: a decade on. *Nat Rev Mol Cell Biol* **8**: 947-956.
Germain P, Staels B, Dacquet C, Spedding M, Laudet V. 2006. Overview of nomenclature of nuclear receptors. *Pharmacol Rev* **58**: 685-704.
Gervois P, Vu-Dac N, Kleemann R, Kockx M, Dubois G, Laine B, Kosykh V, Fruchart JC, Kooistra T, Staels B. 2001. Negative regulation of human fibrinogen gene expression by peroxisome proliferator-activated receptor alpha agonists via inhibition of CCAAT box/enhancer-binding protein beta. *J Biol Chem* **276**: 33471-33477.
Geyeregger R, Shehata M, Zeyda M, Kiefer FW, Stuhlmeier KM, Porpaczy E, Zlabinger GJ, Jager U, Stulnig TM. 2009. Liver X receptors interfere with cytokine-induced proliferation and cell survival in normal and leukemic lymphocytes. *J Leukoc Biol* **86**: 1039-1048.
Geyeregger R, Zeyda M, Bauer W, Kriehuber E, Saemann MD, Zlabinger GJ, Maurer D, Stulnig TM. 2007. Liver X receptors regulate dendritic cell phenotype and function through blocked induction of the actin-bundling protein fascin. *Blood* **109**: 4288-4295.
Ghisletti S, Huang W, Ogawa S, Pascual G, Lin ME, Willson TM, Rosenfeld MG, Glass CK. 2007. Parallel SUMOylation-dependent pathways mediate gene- and signal-specific transrepression by LXRs and PPARgamma. *Mol Cell* **25**: 57-70.
Giguere V. 1999. Orphan nuclear receptors: from gene to function. *Endocr Rev* **20**: 689-725.
Glass CK, Saijo K. Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells. *Nat Rev Immunol* **10**: 365-376.
Goo YH, Sohn YC, Kim DH, Kim SW, Kang MJ, Jung DJ, Kwak E, Barlev NA, Berger SL, Chow VT et al. 2003. Activating signal cointegrator 2 belongs to a novel steady-state complex that contains a subset of trithorax group proteins. *Mol Cell Biol* **23**: 140-149.
Gronemeyer H, Gustafsson JA, Laudet V. 2004. Principles for modulation of the nuclear receptor superfamily. *Nat Rev Drug Discov* **3**: 950-964.
Gruys E, Toussaint MJ, Niewold TA, Koopmans SJ. 2005. Acute phase reaction and acute phase proteins. *J Zhejiang Univ Sci B* **6**: 1045-1056.
Guenther MG, Lane WS, Fischle W, Verdin E, Lazar MA, Shiekhattar R. 2000. A core SMRT corepressor complex containing HDAC3 and TBL1, a WD40-repeat protein linked to deafness. *Genes Dev* **14**: 1048-1057.
Hall JM, McDonnell DP. 2007. The molecular mechanisms underlying the proinflammatory actions of thiazolidinediones in human macrophages. *Mol Endocrinol* **21**: 1756-1768.

Hargreaves DC, Horng T, Medzhitov R. 2009. Control of inducible gene expression by signal-dependent transcriptional elongation. *Cell* **138**: 129-145.
Heery DM, Kalkhoven E, Hoare S, Parker MG. 1997. A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature* **387**: 733-736.
Heintzman ND, Hon GC, Hawkins RD, Kheradpour P, Stark A, Harp LF, Ye Z, Lee LK, Stuart RK, Ching CW et al. 2009. Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature* **459**: 108-112.
Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, Barrera LO, Van Calcar S, Qu C, Ching KA et al. 2007. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat Genet* **39**: 311-318.
Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, Cheng JX, Murre C, Singh H, Glass CK. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* **38**: 576-589.
Heinzel T, Lavinsky RM, Mullen TM, Soderstrom M, Laherty CD, Torchia J, Yang WM, Brard G, Ngo SD, Davie JR et al. 1997. A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. *Nature* **387**: 43-48.
Hermiston ML, Gordon JI. 1995. Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. *Science* **270**: 1203-1207.
Honzumi S, Shima A, Hiroshima A, Koieyama T, Ubukata N, Terasaka N. LXRalpha regulates human CETP expression in vitro and in transgenic mice. *Atherosclerosis* **212**: 139-145.
Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R, Ryan A, Kamei Y, Soderstrom M, Glass CK et al. 1995. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* **377**: 397-404.
Hu X, Lazar MA. 1999. The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors. *Nature* **402**: 93-96.
Hu X, Li S, Wu J, Xia C, Lala DS. 2003. Liver X receptors interact with corepressors to regulate gene expression. *Mol Endocrinol* **17**: 1019-1026.
Huang W, Ghisletti S, Perissi V, Rosenfeld MG, Glass CK. 2009. Transcriptional integration of TLR2 and TLR4 signaling at the NCoR derepression checkpoint. *Mol Cell* **35**: 48-57.
Huang W, Ghisletti S, Saijo K, Gandhi M, Aouadi M, Tesz GJ, Zhang DX, Yao J, Czech MP, Goode BL et al. 2011. Coronin 2A mediates actin-dependent de-repression of inflammatory response genes. *Nature* **470**: 414-418.
Hue S, Ahern P, Buonocore S, Kullberg MC, Cua DJ, McKenzie BS, Powrie F, Maloy KJ. 2006. Interleukin-23 drives innate and T cell-mediated intestinal inflammation. *J Exp Med* **203**: 2473-2483.
Huuskonen J, Fielding PE, Fielding CJ. 2004. Role of p160 coactivator complex in the activation of liver X receptor. *Arterioscler Thromb Vasc Biol* **24**: 703-708.
Ishii KJ, Koyama S, Nakagawa A, Coban C, Akira S. 2008. Host innate immune receptors and beyond: making sense of microbial infections. *Cell Host Microbe* **3**: 352-363.
Jahangiri A. 2010. High-density lipoprotein and the acute phase response. *Curr Opin Endocrinol Diabetes Obes* **17**: 156-160.

Janowski BA, Grogan MJ, Jones SA, Wisely GB, Kliewer SA, Corey EJ, Mangelsdorf DJ. 1999. Structural requirements of ligands for the oxysterol liver X receptors LXRalpha and LXRbeta. *Proc Natl Acad Sci U S A* **96**: 266-271.

Jiang ZY, Parini P, Eggertsen G, Davis MA, Hu H, Suo GJ, Zhang SD, Rudel LL, Han TQ, Einarsson C. 2008. Increased expression of LXR alpha, ABCG5, ABCG8, and SR-BI in the liver from normolipidemic, nonobese Chinese gallstone patients. *J Lipid Res* **49**: 464-472.

Jin DY, Teramoto H, Giam CZ, Chun RF, Gutkind JS, Jeang KT. 1997. A human suppressor of c-Jun N-terminal kinase 1 activation by tumor necrosis factor alpha. *J Biol Chem* **272**: 25816-25823.

Joseph SB, Bradley MN, Castrillo A, Bruhn KW, Mak PA, Pei L, Hogenesch J, O'Connell R M, Cheng G, Saez E et al. 2004. LXR-dependent gene expression is important for macrophage survival and the innate immune response. *Cell* **119**: 299-309.

Joseph SB, Castrillo A, Laffitte BA, Mangelsdorf DJ, Tontonoz P. 2003. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. *Nat Med* **9**: 213-219.

Joseph SB, McKilligin E, Pei L, Watson MA, Collins AR, Laffitte BA, Chen M, Noh G, Goodman J, Hagger GN et al. 2002. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc Natl Acad Sci U S A* **99**: 7604-7609.

Ju BG, Lunyak VV, Perissi V, Garcia-Bassets I, Rose DW, Glass CK, Rosenfeld MG. 2006. A topoisomerase IIbeta-mediated dsDNA break required for regulated transcription. *Science* **312**: 1798-1802.

Kato S, Yokoyama A, Fujiki R. Nuclear receptor coregulators merge transcriptional coregulation with epigenetic regulation. *Trends Biochem Sci*.

Kennedy MA, Barrera GC, Nakamura K, Baldan A, Tarr P, Fishbein MC, Frank J, Francone OL, Edwards PA. 2005. ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. *Cell Metab* **1**: 121-131.

Kennedy MA, Venkateswaran A, Tarr PT, Xenarios I, Kudoh J, Shimizu N, Edwards PA. 2001. Characterization of the human ABCG1 gene: liver X receptor activates an internal promoter that produces a novel transcript encoding an alternative form of the protein. *J Biol Chem* **276**: 39438-39447.

Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C. 2004. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. *J Lipid Res* **45**: 1169-1196.

Kim DH, Lee J, Lee B, Lee JW. 2009a. ASCOM controls farnesoid X receptor transactivation through its associated histone H3 lysine 4 methyltransferase activity. *Mol Endocrinol* **23**: 1556-1562.

Kim GH, Park K, Yeom SY, Lee KJ, Kim G, Ko J, Rhee DK, Kim YH, Lee HK, Kim HW et al. 2009b. Characterization of ASC-2 as an antiatherogenic transcriptional coactivator of liver X receptors in macrophages. *Mol Endocrinol* **23**: 966-974.

Kim SW, Park K, Kwak E, Choi E, Lee S, Ham J, Kang H, Kim JM, Hwang SY, Kong YY et al. 2003. Activating signal cointegrator 2 required for liver lipid metabolism mediated by liver X receptors in mice. *Mol Cell Biol* **23**: 3583-3592.

King VL, Hatch NW, Chan HW, de Beer MC, de Beer FC, Tannock LR. 2010. A murine model of obesity with accelerated atherosclerosis. *Obesity (Silver Spring)* **18**: 35-41.

Kisilevsky R, Tam SP. 2003. Macrophage cholesterol efflux and the active domains of serum amyloid A 2.1. *J Lipid Res* **44**: 2257-2269.

Kleemann R, Verschuren L, de Rooij BJ, Lindeman J, de Maat MM, Szalai AJ, Princen HM, Kooistra T. 2004. Evidence for anti-inflammatory activity of statins and PPARalpha activators in human C-reactive protein transgenic mice in vivo and in cultured human hepatocytes in vitro. *Blood* **103**: 4188-4194.

Klucken J, Buchler C, Orso E, Kaminski WE, Porsch-Ozcurumez M, Liebisch G, Kapinsky M, Diederich W, Drobnik W, Dean M et al. 2000. ABCG1 (ABC8), the human homolog of the Drosophila white gene, is a regulator of macrophage cholesterol and phospholipid transport. *Proc Natl Acad Sci U S A* **97**: 817-822.

Koldamova RP, Lefterov IM, Staufenbiel M, Wolfe D, Huang S, Glorioso JC, Walter M, Roth MG, Lazo JS. 2005. The liver X receptor ligand T0901317 decreases amyloid beta production in vitro and in a mouse model of Alzheimer's disease. *J Biol Chem* **280**: 4079-4088.

Korf H, Vander Beken S, Romano M, Steffensen KR, Stijlemans B, Gustafsson JA, Grooten J, Huygen K. 2009. Liver X receptors contribute to the protective immune response against Mycobacterium tuberculosis in mice. *J Clin Invest* **119**: 1626-1637.

Kuang SQ, Liao L, Zhang H, Pereira FA, Yuan Y, DeMayo FJ, Ko L, Xu J. 2002. Deletion of the cancer-amplified coactivator AIB3 results in defective placentation and embryonic lethality. *J Biol Chem* **277**: 45356-45360.

Laffitte BA, Joseph SB, Chen M, Castrillo A, Repa J, Wilpitz D, Mangelsdorf D, Tontonoz P. 2003. The phospholipid transfer protein gene is a liver X receptor target expressed by macrophages in atherosclerotic lesions. *Mol Cell Biol* **23**: 2182-2191.

Laffitte BA, Repa JJ, Joseph SB, Wilpitz DC, Kast HR, Mangelsdorf DJ, Tontonoz P. 2001. LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes. *Proc Natl Acad Sci U S A* **98**: 507-512.

Lee JH, Park SM, Kim OS, Lee CS, Woo JH, Park SJ, Joe EH, Jou I. 2009. Differential SUMOylation of LXRalpha and LXRbeta mediates transrepression of STAT1 inflammatory signaling in IFN-gamma-stimulated brain astrocytes. *Mol Cell* **35**: 806-817.

Lee S, Lee DK, Dou Y, Lee J, Lee B, Kwak E, Kong YY, Lee SK, Roeder RG, Lee JW. 2006. Coactivator as a target gene specificity determinant for histone H3 lysine 4 methyltransferases. *Proc Natl Acad Sci U S A* **103**: 15392-15397.

Lee S, Lee J, Lee SK, Lee JW. 2008. Activating signal cointegrator-2 is an essential adaptor to recruit histone H3 lysine 4 methyltransferases MLL3 and MLL4 to the liver X receptors. *Mol Endocrinol* **22**: 1312-1319.

Lee SK, Jung SY, Kim YS, Na SY, Lee YC, Lee JW. 2001. Two distinct nuclear receptor-interaction domains and CREB-binding protein-dependent transactivation function of activating signal cointegrator-2. *Mol Endocrinol* **15**: 241-254.

Li G, Biju KC, Xu X, Zhou Q, Chen C, Valente AJ, He W, Reddick RL, Freeman GL, Ahuja SS et al. 2011. Macrophage LXRalpha gene therapy ameliorates atherosclerosis as well as hypertriglyceridemia in LDLR(-/-) mice. *Gene Ther*.

Li J, Wang J, Nawaz Z, Liu JM, Qin J, Wong J. 2000. Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. *EMBO J* **19**: 4342-4350.

Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. 2006. Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol* **24**: 99-146.

Li X, Zhang S, Blander G, Tse JG, Krieger M, Guarente L. 2007. SIRT1 deacetylates and positively regulates the nuclear receptor LXR. *Mol Cell* **28**: 91-106.

Lo Sasso G, Murzilli S, Salvatore L, D'Errico I, Petruzzelli M, Conca P, Jiang ZY, Calabresi L, Parini P, Moschetta A. Intestinal specific LXR activation stimulates reverse cholesterol transport and protects from atherosclerosis. *Cell Metab* **12**: 187-193.

Loftus EV, Jr. 2004. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* **126**: 1504-1517.

Luo Y, Tall AR. 2000. Sterol upregulation of human CETP expression in vitro and in transgenic mice by an LXR element. *J Clin Invest* **105**: 513-520.

Lupien M, Eeckhoute J, Meyer CA, Wang Q, Zhang Y, Li W, Carroll JS, Liu XS, Brown M. 2008. FoxA1 translates epigenetic signatures into enhancer-driven lineage-specific transcription. *Cell* **132**: 958-970.

Mahajan MA, Das S, Zhu H, Tomic-Canic M, Samuels HH. 2004. The nuclear hormone receptor coactivator NRC is a pleiotropic modulator affecting growth, development, apoptosis, reproduction, and wound repair. *Mol Cell Biol* **24**: 4994-5004.

Mahajan MA, Samuels HH. 2008. Nuclear receptor coactivator/coregulator NCoA6(NRC) is a pleiotropic coregulator involved in transcription, cell survival, growth and development. *Nucl Recept Signal* **6**: e002.

Mak PA, Kast-Woelbern HR, Anisfeld AM, Edwards PA. 2002. Identification of PLTP as an LXR target gene and apoE as an FXR target gene reveals overlapping targets for the two nuclear receptors. *J Lipid Res* **43**: 2037-2041.

Mansouri RM, Bauge E, Staels B, Gervois P. 2008. Systemic and distal repercussions of liver-specific peroxisome proliferator-activated receptor-alpha control of the acute-phase response. *Endocrinology* **149**: 3215-3223.

Marcil M, Brooks-Wilson A, Clee SM, Roomp K, Zhang LH, Yu L, Collins JA, van Dam M, Molhuizen HO, Loubster O et al. 1999. Mutations in the ABC1 gene in familial HDL deficiency with defective cholesterol efflux. *Lancet* **354**: 1341-1346.

Massague J, Seoane J, Wotton D. 2005. Smad transcription factors. *Genes Dev* **19**: 2783-2810.

Matsuda T, Yamamoto T, Muraguchi A, Saatcioglu F. 2001. Cross-talk between transforming growth factor-beta and estrogen receptor signaling through Smad3. *J Biol Chem* **276**: 42908-42914.

McGillicuddy FC, de la Llera Moya M, Hinkle CC, Joshi MR, Chiquoine EH, Billheimer JT, Rothblat GH, Reilly MP. 2009. Inflammation impairs reverse cholesterol transport in vivo. *Circulation* **119**: 1135-1145.

Metzger E, Wissmann M, Yin N, Muller JM, Schneider R, Peters AH, Gunther T, Buettner R, Schule R. 2005. LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature* **437**: 436-439.

Molteni V, Li X, Nabakka J, Liang F, Wityak J, Koder A, Vargas L, Romeo R, Mitro N, Mak PA et al. 2007. N-Acylthiadiazolines, a new class of liver X receptor agonists with selectivity for LXRbeta. *J Med Chem* **50**: 4255-4259.

Moshage H. 1997. Cytokines and the hepatic acute phase response. *J Pathol* **181**: 257-266.

Nagy L, Kao HY, Chakravarti D, Lin RJ, Hassig CA, Ayer DE, Schreiber SL, Evans RM. 1997. Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell* **89**: 373-380.

Naik SU, Wang X, Da Silva JS, Jaye M, Macphee CH, Reilly MP, Billheimer JT, Rothblat GH, Rader DJ. 2006. Pharmacological activation of liver X receptors promotes reverse cholesterol transport in vivo. *Circulation* **113**: 90-97.

Oberkofler H, Schraml E, Krempler F, Patsch W. 2003. Potentiation of liver X receptor transcriptional activity by peroxisome-proliferator-activated receptor gamma co-activator 1 alpha. *Biochem J* **371**: 89-96.

Oberoi J, Fairall L, Watson PJ, Yang JC, Czimmerer Z, Kampmann T, Goult BT, Greenwood JA, Gooch JT, Kallenberger BC et al. Structural basis for the assembly of the SMRT/NCoR core transcriptional repression machinery. *Nat Struct Mol Biol* **18**: 177-184.

Ogawa S, Lozach J, Benner C, Pascual G, Tangirala RK, Westin S, Hoffmann A, Subramaniam S, David M, Rosenfeld MG et al. 2005. Molecular determinants of crosstalk between nuclear receptors and toll-like receptors. *Cell* **122**: 707-721.

Ogawa S, Lozach J, Jepsen K, Sawka-Verhelle D, Perissi V, Sasik R, Rose DW, Johnson RS, Rosenfeld MG, Glass CK. 2004. A nuclear receptor corepressor transcriptional checkpoint controlling activator protein 1-dependent gene networks required for macrophage activation. *Proc Natl Acad Sci U S A* **101**: 14461-14466.

Onate SA, Tsai SY, Tsai MJ, O'Malley BW. 1995. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* **270**: 1354-1357.

Ong CT, Corces VG. Enhancer function: new insights into the regulation of tissue-specific gene expression. *Nat Rev Genet* **12**: 283-293.

Oram JF, Vaughan AM. 2006. ATP-Binding cassette cholesterol transporters and cardiovascular disease. *Circ Res* **99**: 1031-1043.

Panousis CG, Evans G, Zuckerman SH. 2001. TGF-beta increases cholesterol efflux and ABC-1 expression in macrophage-derived foam cells: opposing the effects of IFN-gamma. *J Lipid Res* **42**: 856-863.

Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, Rose DW, Willson TM, Rosenfeld MG, Glass CK. 2005. A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature* **437**: 759-763.

Pascual G, Glass CK. 2006. Nuclear receptors versus inflammation: mechanisms of transrepression. *Trends Endocrinol Metab* **17**: 321-327.

Peet DJ, Turley SD, Ma W, Janowski BA, Lobaccaro JM, Hammer RE, Mangelsdorf DJ. 1998. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. *Cell* **93**: 693-704.

Peng YC, Breiding DE, Sverdrup F, Richard J, Androphy EJ. 2000. AMF-1/Gps2 binds p300 and enhances its interaction with papillomavirus E2 proteins. *J Virol* **74**: 5872-5879.

Peng YC, Kuo F, Breiding DE, Wang YF, Mansur CP, Androphy EJ. 2001. AMF1 (GPS2) modulates p53 transactivation. *Mol Cell Biol* **21**: 5913-5924.

Perissi V, Aggarwal A, Glass CK, Rose DW, Rosenfeld MG. 2004. A corepressor/coactivator exchange complex required for transcriptional activation by nuclear receptors and other regulated transcription factors. *Cell* **116**: 511-526.

Perissi V, Jepsen K, Glass CK, Rosenfeld MG. Deconstructing repression: evolving models of co-repressor action. *Nat Rev Genet* **11**: 109-123.

Perissi V, Scafoglio C, Zhang J, Ohgi KA, Rose DW, Glass CK, Rosenfeld MG. 2008. TBL1 and TBLR1 phosphorylation on regulated gene promoters overcomes dual CtBP and NCoR/SMRT transcriptional repression checkpoints. *Mol Cell* **29**: 755-766.

Perissi V, Staszewski LM, McInerney EM, Kurokawa R, Krones A, Rose DW, Lambert MH, Milburn MV, Glass CK, Rosenfeld MG. 1999. Molecular

determinants of nuclear receptor-corepressor interaction. *Genes Dev* **13**: 3198-3208.
Poitou C, Divoux A, Faty A, Tordjman J, Hugol D, Aissat A, Keophiphath M, Henegar C, Commans S, Clement K. 2009. Role of serum amyloid a in adipocyte-macrophage cross talk and adipocyte cholesterol efflux. *J Clin Endocrinol Metab* **94**: 1810-1817.
Poukka H, Karvonen U, Janne OA, Palvimo JJ. 2000. Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). *Proc Natl Acad Sci U S A* **97**: 14145-14150.
Rader DJ. 2006. Molecular regulation of HDL metabolism and function: implications for novel therapies. *J Clin Invest* **116**: 3090-3100.
Rader DJ, Daugherty A. 2008. Translating molecular discoveries into new therapies for atherosclerosis. *Nature* **451**: 904-913.
Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. 2004. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* **118**: 229-241.
Ramakers JD, Verstege MI, Thuijls G, Te Velde AA, Mensink RP, Plat J. 2007. The PPARgamma agonist rosiglitazone impairs colonic inflammation in mice with experimental colitis. *J Clin Immunol* **27**: 275-283.
Renaud JP, Rochel N, Ruff M, Vivat V, Chambon P, Gronemeyer H, Moras D. 1995. Crystal structure of the RAR-gamma ligand-binding domain bound to all-trans retinoic acid. *Nature* **378**: 681-689.
Repa JJ, Berge KE, Pomajzl C, Richardson JA, Hobbs H, Mangelsdorf DJ. 2002. Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors alpha and beta. *J Biol Chem* **277**: 18793-18800.
Repa JJ, Turley SD, Lobaccaro JA, Medina J, Li L, Lustig K, Shan B, Heyman RA, Dietschy JM, Mangelsdorf DJ. 2000. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science* **289**: 1524-1529.
Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. 1998. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* **391**: 79-82.
Robertson AK, Rudling M, Zhou X, Gorelik L, Flavell RA, Hansson GK. 2003. Disruption of TGF-beta signaling in T cells accelerates atherosclerosis. *J Clin Invest* **112**: 1342-1350.
Rosen IMaFS. 2000. The immune system. *The New England Journal of Medicine* **343**.
Rosenfeld MG, Lunyak VV, Glass CK. 2006. Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response. *Genes Dev* **20**: 1405-1428.
Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, Deleuze JF, Brewer HB, Duverger N, Denefle P et al. 1999. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. *Nat Genet* **22**: 352-355.
Sabol SL, Brewer HB, Jr., Santamarina-Fojo S. 2005. The human ABCG1 gene: identification of LXR response elements that modulate expression in macrophages and liver. *J Lipid Res* **46**: 2151-2167.
Sanchez-Hidalgo M, Martin AR, Villegas I, de la Lastra CA. 2007. Rosiglitazone, a PPARgamma ligand, modulates signal transduction pathways during the development of acute TNBS-induced colitis in rats. *Eur J Pharmacol* **562**: 247-258.
Sanyal S, Bavner A, Haroniti A, Nilsson LM, Lundasen T, Rehnmark S, Witt MR, Einarsson C, Talianidis I, Gustafsson JA et al. 2007. Involvement of corepressor complex subunit GPS2 in transcriptional pathways governing human bile acid biosynthesis. *Proc Natl Acad Sci U S A* **104**: 15665-15670.

Schultz JR, Tu H, Luk A, Repa JJ, Medina JC, Li L, Schwendner S, Wang S, Thoolen M, Mangelsdorf DJ et al. 2000. Role of LXRs in control of lipogenesis. *Genes Dev* **14**: 2831-2838.

Schuster GU, Parini P, Wang L, Alberti S, Steffensen KR, Hansson GK, Angelin B, Gustafsson JA. 2002. Accumulation of foam cells in liver X receptor-deficient mice. *Circulation* **106**: 1147-1153.

Schwartz K, Lawn RM, Wade DP. 2000. ABC1 gene expression and ApoA-I-mediated cholesterol efflux are regulated by LXR. *Biochem Biophys Res Commun* **274**: 794-802.

Shah YM, Ma X, Morimura K, Kim I, Gonzalez FJ. 2007a. Pregnane X receptor activation ameliorates DSS-induced inflammatory bowel disease via inhibition of NF-kappaB target gene expression. *Am J Physiol Gastrointest Liver Physiol* **292**: G1114-1122.

Shah YM, Morimura K, Gonzalez FJ. 2007b. Expression of peroxisome proliferator-activated receptor-gamma in macrophage suppresses experimentally induced colitis. *Am J Physiol Gastrointest Liver Physiol* **292**: G657-666.

Shen Q, Bai Y, Chang KC, Wang Y, Burris TP, Freedman LP, Thompson CC, Nagpal S. Liver X receptor-retinoid X receptor (LXR-RXR) heterodimer cistrome reveals co-ordination of LXR and AP1 signaling in keratinocytes. *J Biol Chem*.

Shibata N, Glass CK. 2010. Macrophages, oxysterols and atherosclerosis. *Circ J* **74**: 2045-2051.

Shinar DM, Endo N, Rutledge SJ, Vogel R, Rodan GA, Schmidt A. 1994. NER, a new member of the gene family encoding the human steroid hormone nuclear receptor. *Gene* **147**: 273-276.

Smoak K, Madenspacher J, Jeyaseelan S, Williams B, Dixon D, Poch KR, Nick JA, Worthen GS, Fessler MB. 2008. Effects of liver X receptor agonist treatment on pulmonary inflammation and host defense. *J Immunol* **180**: 3305-3312.

Song C, Kokontis JM, Hiipakka RA, Liao S. 1994. Ubiquitous receptor: a receptor that modulates gene activation by retinoic acid and thyroid hormone receptors. *Proc Natl Acad Sci U S A* **91**: 10809-10813.

Song CZ, Tian X, Gelehrter TD. 1999. Glucocorticoid receptor inhibits transforming growth factor-beta signaling by directly targeting the transcriptional activation function of Smad3. *Proc Natl Acad Sci U S A* **96**: 11776-11781.

Spain BH, Bowdish KS, Pacal AR, Staub SF, Koo D, Chang CY, Xie W, Colicelli J. 1996. Two human cDNAs, including a homolog of Arabidopsis FUS6 (COP11), suppress G-protein- and mitogen-activated protein kinase-mediated signal transduction in yeast and mammalian cells. *Mol Cell Biol* **16**: 6698-6706.

Sparrow CP, Baffic J, Lam MH, Lund EG, Adams AD, Fu X, Hayes N, Jones AB, Macnaul KL, Ondeyka J et al. 2002. A potent synthetic LXR agonist is more effective than cholesterol loading at inducing ABCA1 mRNA and stimulating cholesterol efflux. *J Biol Chem* **277**: 10021-10027.

Stockert J, Adhikary T, Kaddatz K, Finkernagel F, Meissner W, Muller-Brusselbach S, Muller R. 2011. Reverse crosstalk of TGFbeta and PPARbeta/delta signaling identified by transcriptional profiling. *Nucleic Acids Res* **39**: 119-131.

Strober W, Murray PJ, Kitani A, Watanabe T. 2006. Signalling pathways and molecular interactions of NOD1 and NOD2. *Nat Rev Immunol* **6**: 9-20.

Sun Y, Yao J, Kim TW, Tall AR. 2003. Expression of liver X receptor target genes decreases cellular amyloid beta peptide secretion. *J Biol Chem* **278**: 27688-27694.

Surapureddi S, Rana R, Goldstein JA. NCOA6 differentially regulates the expression of the CYP2C9 and CYP3A4 genes. *Pharmacol Res*.

Surapureddi S, Rana R, Reddy JK, Goldstein JA. 2008. Nuclear receptor coactivator 6 mediates the synergistic activation of human cytochrome P-450 2C9 by the constitutive androstane receptor and hepatic nuclear factor-4alpha. *Mol Pharmacol* **74**: 913-923.

Tam SP, Ancsin JB, Tan R, Kisilevsky R. 2005. Peptides derived from serum amyloid A prevent, and reverse, aortic lipid lesions in apoE-/- mice. *J Lipid Res* **46**: 2091-2101.

Tam SP, Flexman A, Hulme J, Kisilevsky R. 2002. Promoting export of macrophage cholesterol: the physiological role of a major acute-phase protein, serum amyloid A 2.1. *J Lipid Res* **43**: 1410-1420.

Tam SP, Kisilevsky R, Ancsin JB. 2008. Acute-phase-HDL remodeling by heparan sulfate generates a novel lipoprotein with exceptional cholesterol efflux activity from macrophages. *PLoS One* **3**: e3867.

Tangirala RK, Bischoff ED, Joseph SB, Wagner BL, Walczak R, Laffitte BA, Daige CL, Thomas D, Heyman RA, Mangelsdorf DJ et al. 2002. Identification of macrophage liver X receptors as inhibitors of atherosclerosis. *Proc Natl Acad Sci U S A* **99**: 11896-11901.

Teboul M, Enmark E, Li Q, Wikstrom AC, Pelto-Huikko M, Gustafsson JA. 1995. OR-1, a member of the nuclear receptor superfamily that interacts with the 9-cis-retinoic acid receptor. *Proc Natl Acad Sci U S A* **92**: 2096-2100.

ten Dijke P, Hill CS. 2004. New insights into TGF-beta-Smad signalling. *Trends Biochem Sci* **29**: 265-273.

Tillett WS, Francis T. 1930. Serological Reactions in Pneumonia with a Non-Protein Somatic Fraction of Pneumococcus. *J Exp Med* **52**: 561-571.

Treuter E, Venteclef N. Transcriptional control of metabolic and inflammatory pathways by nuclear receptor SUMOylation. *Biochim Biophys Acta*.

Unno A, Takada I, Takezawa S, Oishi H, Baba A, Shimizu T, Tokita A, Yanagisawa J, Kato S. 2005. TRRAP as a hepatic coactivator of LXR and FXR function. *Biochem Biophys Res Commun* **327**: 933-938.

Valledor AF, Hsu LC, Ogawa S, Sawka-Verhelle D, Karin M, Glass CK. 2004. Activation of liver X receptors and retinoid X receptors prevents bacterial-induced macrophage apoptosis. *Proc Natl Acad Sci U S A* **101**: 17813-17818.

Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. 2009. The bile acid receptor FXR is a modulator of intestinal innate immunity. *J Immunol* **183**: 6251-6261.

Venkateswaran A, Laffitte BA, Joseph SB, Mak PA, Wilpitz DC, Edwards PA, Tontonoz P. 2000. Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXR alpha. *Proc Natl Acad Sci U S A* **97**: 12097-12102.

Venteclef N, Delerive P. 2007. Interleukin-1 receptor antagonist induction as an additional mechanism for liver receptor homolog-1 to negatively regulate the hepatic acute phase response. *J Biol Chem* **282**: 4393-4399.

Venteclef N, Smith JC, Goodwin B, Delerive P. 2006. Liver receptor homolog 1 is a negative regulator of the hepatic acute-phase response. *Mol Cell Biol* **26**: 6799-6807.

Vijay-Kumar M, Sanders CJ, Taylor RT, Kumar A, Aitken JD, Sitaraman SV, Neish AS, Uematsu S, Akira S, Williams IR et al. 2007. Deletion of TLR5 results in spontaneous colitis in mice. *J Clin Invest* **117**: 3909-3921.

Villablanca EJ, Raccosta L, Zhou D, Fontana R, Maggioni D, Negro A, Sanvito F, Ponzoni M, Valentinis B, Bregni M et al. Tumor-mediated liver X receptor-alpha activation inhibits CC chemokine receptor-7 expression on dendritic cells and dampens antitumor responses. *Nat Med* **16**: 98-105.

Voegel JJ, Heine MJ, Zechel C, Chambon P, Gronemeyer H. 1996. TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors. *Embo J* **15**: 3667-3675.

Wagner BL, Valledor AF, Shao G, Daige CL, Bischoff ED, Petrowski M, Jepsen K, Baek SH, Heyman RA, Rosenfeld MG et al. 2003. Promoter-specific roles for liver X receptor/corepressor complexes in the regulation of ABCA1 and SREBP1 gene expression. *Mol Cell Biol* **23**: 5780-5789.

Wang N, Lan D, Chen W, Matsuura F, Tall AR. 2004. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. *Proc Natl Acad Sci U S A* **101**: 9774-9779.

Wang X, Collins HL, Ranalletta M, Fuki IV, Billheimer JT, Rothblat GH, Tall AR, Rader DJ. 2007. Macrophage ABCA1 and ABCG1, but not SR-BI, promote macrophage reverse cholesterol transport in vivo. *J Clin Invest* **117**: 2216-2224.

Wang Y, Kinzie E, Berger FG, Lim SK, Baumann H. 2001. Haptoglobin, an inflammation-inducible plasma protein. *Redox Rep* **6**: 379-385.

Warnmark A, Treuter E, Wright AP, Gustafsson JA. 2003. Activation functions 1 and 2 of nuclear receptors: molecular strategies for transcriptional activation. *Mol Endocrinol* **17**: 1901-1909.

Wiebel FF, Steffensen KR, Treuter E, Feltkamp D, Gustafsson JA. 1999. Ligand-independent coregulator recruitment by the triply activatable OR1/retinoid X receptor-alpha nuclear receptor heterodimer. *Mol Endocrinol* **13**: 1105-1118.

Williams S, Bledsoe RK, Collins JL, Boggs S, Lambert MH, Miller AB, Moore J, McKee DD, Moore L, Nichols J et al. 2003. X-ray crystal structure of the liver X receptor beta ligand binding domain: regulation by a histidine-tryptophan switch. *J Biol Chem* **278**: 27138-27143.

Willy PJ, Umesono K, Ong ES, Evans RM, Heyman RA, Mangelsdorf DJ. 1995. LXR, a nuclear receptor that defines a distinct retinoid response pathway. *Genes Dev* **9**: 1033-1045.

Wilund KR, Yu L, Xu F, Hobbs HH, Cohen JC. 2004. High-level expression of ABCG5 and ABCG8 attenuates diet-induced hypercholesterolemia and atherosclerosis in Ldlr-/- mice. *J Lipid Res* **45**: 1429-1436.

Wissmann M, Yin N, Muller JM, Greschik H, Fodor BD, Jenuwein T, Vogler C, Schneider R, Gunther T, Buettner R et al. 2007. Cooperative demethylation by JMJD2C and LSD1 promotes androgen receptor-dependent gene expression. *Nat Cell Biol* **9**: 347-353.

Yamane K, Toumazou C, Tsukada Y, Erdjument-Bromage H, Tempst P, Wong J, Zhang Y. 2006. JHDM2A, a JmjC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor. *Cell* **125**: 483-495.

Yang RZ, Lee MJ, Hu H, Pollin TI, Ryan AS, Nicklas BJ, Snitker S, Horenstein RB, Hull K, Goldberg NH et al. 2006. Acute-phase serum amyloid A: an inflammatory adipokine and potential link between obesity and its metabolic complications. *PLoS Med* **3**: e287.

Yasuda T, Grillot D, Billheimer JT, Briand F, Delerive P, Huet S, Rader DJ. 2010. Tissue-specific liver X receptor activation promotes macrophage reverse cholesterol transport in vivo. *Arterioscler Thromb Vasc Biol* **30**: 781-786.

Yoon HG, Chan DW, Huang ZQ, Li J, Fondell JD, Qin J, Wong J. 2003. Purification and functional characterization of the human N-CoR complex: the roles of HDAC3, TBL1 and TBLR1. *EMBO J* **22**: 1336-1346.

Yoon HG, Choi Y, Cole PA, Wong J. 2005. Reading and function of a histone code involved in targeting corepressor complexes for repression. *Mol Cell Biol* **25**: 324-335.

Yu L, York J, von Bergmann K, Lutjohann D, Cohen JC, Hobbs HH. 2003. Stimulation of cholesterol excretion by the liver X receptor agonist requires ATP-binding cassette transporters G5 and G8. *J Biol Chem* **278**: 15565-15570.

Yvan-Charvet L, Ranalletta M, Wang N, Han S, Terasaka N, Li R, Welch C, Tall AR. 2007. Combined deficiency of ABCA1 and ABCG1 promotes foam cell accumulation and accelerates atherosclerosis in mice. *J Clin Invest* **117**: 3900-3908.

Yvan-Charvet L, Wang N, Tall AR. Role of HDL, ABCA1, and ABCG1 transporters in cholesterol efflux and immune responses. *Arterioscler Thromb Vasc Biol* **30**: 139-143.

Zambon A, Gervois P, Pauletto P, Fruchart JC, Staels B. 2006. Modulation of hepatic inflammatory risk markers of cardiovascular diseases by PPAR-alpha activators: clinical and experimental evidence. *Arterioscler Thromb Vasc Biol* **26**: 977-986.

Zelcer N, Khanlou N, Clare R, Jiang Q, Reed-Geaghan EG, Landreth GE, Vinters HV, Tontonoz P. 2007. Attenuation of neuroinflammation and Alzheimer's disease pathology by liver x receptors. *Proc Natl Acad Sci U S A* **104**: 10601-10606.

Zelcer N, Tontonoz P. 2006. Liver X receptors as integrators of metabolic and inflammatory signaling. *J Clin Invest* **116**: 607-614.

Zhang J, Kalkum M, Chait BT, Roeder RG. 2002. The N-CoR-HDAC3 nuclear receptor corepressor complex inhibits the JNK pathway through the integral subunit GPS2. *Mol Cell* **9**: 611-623.

Zhao Y, He X, Shi X, Huang C, Liu J, Zhou S, Heng CK. 2010. Association between serum amyloid A and obesity: a meta-analysis and systematic review. *Inflamm Res* **59**: 323-334.

Zhu X, Lee JY, Timmins JM, Brown JM, Boudyguina E, Mulya A, Gebre AK, Willingham MC, Hiltbold EM, Mishra N et al. 2008. Increased cellular free cholesterol in macrophage-specific Abca1 knock-out mice enhances pro-inflammatory response of macrophages. *J Biol Chem* **283**: 22930-22941.

Zuercher WJ, Buckholz RG, Campobasso N, Collins JL, Galardi CM, Gampe RT, Hyatt SM, Merrihew SL, Moore JT, Oplinger JA et al. 2010. Discovery of tertiary sulfonamides as potent liver X receptor antagonists. *J Med Chem* **53**: 3412-3416.